The role of nicotinic acetylcholine receptors in motivated behaviour

Wright, Victoria

Award date:
2015

Awarding institution:
University of Bath

The role of nicotinic acetylcholine receptors in motivated behaviour

Victoria Louise Wright

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Biology and Biochemistry

Department of Pharmacy and Pharmacology

September 2015

ACKNOWLEDGEMENTS

This work was performed under the supervision of Prof Sue Wonnacott, Dr Chris Bailey, and Prof David Heal with financial support from a BBSRC CASE studentship with RenaSci, Nottingham, UK. All data presented here were generated by myself, with the exception of the autoradiography binding that was done in conjunction with Polymina Georgiou from University of Surrey under the supervision of Dr Alexis Bailey.

My special thanks go to my supervisors who have supported me throughout this process. For technical support I would like to thank the 4SA staff. In particular Martin, Lesley, Jean and Jane for all their patience with the surgical work and for giving me some breaks at the weekends! I would like to thank Jane for all the support with the post-op care and Al for his ingenious solutions to all my technical issues; I really couldn't have done it without you.

Thank you to Jack, Ruquaiya, Chris and Julia – the original office crew. Particularly to Julia, for introducing me to an obsession that has brought me immeasurable pleasure over the last year. The creative outlet has been absolutely invaluable on this journey, and I'm sure it is the reason I still possess some of my marbles! To Annelisa who's addition to any afternoon spent watching yellow dots dart across a screen is a VERY welcome one; and to Matt, the electrophysiology God for so many that grace the Bailey lab (P.S thank you for holding my hand on the plane to INRC!).

For support in this work and all other areas of my life I'm am hugely grateful to my best friend and partner in all things biology related. Thank you for sharing my love of nature, science and general enthusiasm for life. I am forever in debt to my wonderful parents, Tim and Di and increasingly so to my extended family (thank you for funding the Data dress Max!). Finally, to Dave thank you for everything you do for me, one day I hope to return the love and support you have shown me throughout this process.

ABSTRACT

Understanding how memory, learning and reward work in unison to form adaptive and sometime maladaptive behaviour is at the forefront of modern neuroscience. The largest unmet need in treating maladaptive reward learning behaviours such as addiction is maintaining long-term abstinence and preventing relapse after re-exposure to drug-associated cues. Nicotinic acetylcholine receptors (nAChR) have been implicated in responses to drugs of abuse other than nicotine (Rahman *et al.*, 2015) and the aim of this work was to characterise the role of α7 nAChRs in morphine reward learning using conditioned place preference (CPP). The α7 nAChR antagonist methyllycaconitine (MLA) was used to determine if these receptors contribute to specific stages of drug-paired learning, namely acquisition, expression, reconsolidation or reinstatement of morphine-CPP. In 7-8week old C57BL/6J mice MLA (4mg/kg, s.c), given 20 minutes prior to a conditioning dose of morphine (10mg/kg, i.p) or post-test trial, had no effect on the acquisition, reconsolidation or expression of morphine-CPP. However, when given 20 minutes prior to a priming dose of morphine (5mg/kg, i.p), MLA (4mg/kg, s.c) significantly inhibited drug-induced reinstatement. The mechanisms of this effect were investigated using glutamate receptor autoradiography. Changes in 2-Amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) and N-methyl-D-aspartate (NMDA) binding were examined in mice treated with either saline or MLA at morphine reinstatement. There were no significant changes in NMDA receptor binding (using [^{3}H]MK-801) but morphine reinstatement significantly increased [^{3}H]AMPA binding in the CA1/2 of the ventral but not dorsal hippocampus, or in any other brain regions examined (including mPFC, nucleus accumbens, amygdala and VTA). The selective increase in the hippocampus was partially antagonised by MLA, linking α7 nAChR activation to glutamatergic synaptic plasticity in the hippocampus. Intracranial infusions of MLA into the ventral but not the dorsal hippocampus or medial prefrontal cortex blocked reinstatement to morphine-CPP in male Wistar rats.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACh Acetylcholine

AMPA(R) 2-Amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (receptor)

AMG Amygdala

ANOVA Analysis of variance

AuCx Auditory cortex

BF Basal Forebrain

BLA Basolateral amygdala

CA1-3 Cornu ammonis regions 1-3

cAMP cyclic adenosine monophosphate

CNS Central nervous system

CPP Conditioned place preference

CPu Caudate putamen

CREB cAMP response element-binding protein

CS Conditioned stimulus

DA Dopamine

DG Dentate gyrus

dHPC Dorsal hippocampus

DHβE Dihydro-beta-erythroidine

DP Drug-paired

EPSP Excitatory postsynaptic current

fEPSP Field excitatory postsynaptic potential

GABA Gamma-aminobutyric acid

HPC Hippocampus

i.p intraperitoneal

iGLuR Iontropic glutamate receptor

IL Infralimbic

IPSC Inhibitory postsynaptic current

LDT Lateral dorsal tegmental

LTD Long term depression

LTP Long term potentiation

M1-2 Motor cortex

mAChR Muscarinic acetylcholine receptors

MAPK Mitogen-activated protein kinase

mGLuR Metabotropic glutamate receptor

MK-801 Dizocilpine

MLA Methyllycaconitine

mPFC Medial prefrontal cortex

MRI Magnetic resonance imaging

mRNA Messenger ribonucleic acid

MS medial septum

MSN Medium spiny neuron

NaC Nucleus accumbens

nACh(R) Nicotinic acetylcholine (receptor)

NCAM Neural cell adhesion molecule

NMDA N-methyl-D-aspartate (receptor)

PET Positron emission topography

PFC Prefrontal cortex

PKA Protein kinase A

PPT pedunculopontine tegmental nuclei

PrL Prelimbic

PSD Postsynaptic density

s.c Subcutaneous

SC Schaffer collateral

SEM Standard error of the mean

SN substantia nigra

STD Short term depression

TARP Transmembrane AMPA regulatory protein

US Unconditioned Stimulus

VDB vertical limb of the diagonal band of broca

VGCC Voltage-gated calcium channel

vHPC Ventral hippocampus

ViCx Visual cortex

VTA Ventral tegmental area

α-BGT alpha bungratoxin

CHAPTER 1 INTRODUCTION

1.1 Memory, learning and reward

The forefathers of modern psychology, Skinner, Pavlov and Watson played strong roles in defining the terms reward and reinforcement. To Skinner, reinforcement was at the core of understanding behaviour, it formed the strengthening effect that increased the likelihood of certain behaviour. Pavlov and Watson showed the powerful effects of the associations made between stimuli and objects in two infamous studies: that of Pavlov's dogs and Watson's 'little Albert'. The importance of these findings helped shape understanding of this essential behaviour that allows appropriate responding to environmental stimuli which ultimately enhances chances of survival, but can sometimes become maladaptive. Most motile animals show some form of foraging behaviour to locate food, water or a mate, and will actively avoid stimuli that are harmful. These responses are used to determine whether a stimulus is rewarding or aversive to an animal, a reward elicits approach behaviour, whereas punishing stimuli elicits avoidance behaviour (Skinner, 1938). Understanding how memory, learning and reward work in unison to form adaptive and sometimes maladaptive behaviour is still at the forefront of modern neuroscience (For review see White, 1996; Kelley & Berridge, 2002).

Maladaptive reward learning

Drugs of abuse act on the same reward system that evolved to respond to these 'natural rewards'. The desire to self-stimulate this pathway with exogenous compounds is as old as mankind itself, mushrooms and herbs have been gathered for their medicinal properties for thousands of years, but it is thought that the discovery of fermentation in around 6000BC was the first mass scale example purely for its psychological effects. This behaviour is by no means limited to humans, wild animals have been shown to return to flowers producing alcohol containing-nectar (Wiens *et al.*, 2008) and there have even been cases of bears binging on beer (BBC, 2004).

Today drugs of abuse pose more of a public health issue than ever before, illicit drug taking is widespread and appears to be on the rise. A recent drug survey revealed that nearly one in three British adults has taken an illegal substance and a fifth of those still do. Approximately 2 million people, consider themselves to have a problem with drugs, and it is likely that this is a conservative figure due to the nature of self-report studies. Half of these people are either in treatment or no longer use, but there are still 1 million Britons living with an illicit drug addiction ('The Global Drug Survey 2014').

However, the most widely used drugs are legal, and the abuse of these arguably poses an even larger harm both medically and socially (see figure 1.1:Nutt *et al.*, 2010). There are about 10 million adult smokers in the UK, which equates to about a sixth of the population and around 90% of tobacco smokers are thought to be addicted (Benowitz & Hatsukami, 1998; Action on Smoking and Health, 2015). Overall 38 per cent of men and 25 per cent of women exceed the recommended daily benchmark for alcohol consumption (Health and Social Care Information Centre, 2012) and the number of people seeking help for alcohol abuse is on the rise. In 2012, there were 178,247 prescription items prescribed for the treatment of alcohol dependence, this is an increase of 6% on the 2011 figure and 73% from 2013 (Health and Social Care Information Centre, 2012). Understanding of addiction vulnerability is still rudimentary, due the fact that only a minority of drug users progress to a *bona fide* state of drug addiction, suggesting there are complex interactions between genetic and social factors (Baler & Volkow, 2006). Importantly, illicit and licit drugs are thought to have overlapping mechanisms of action. In particular, molecular processes involved in associative learning between drug-associated cues (such as particular individuals or environment) are thought to be important in initiating and maintaining illicit and licit drug taking.

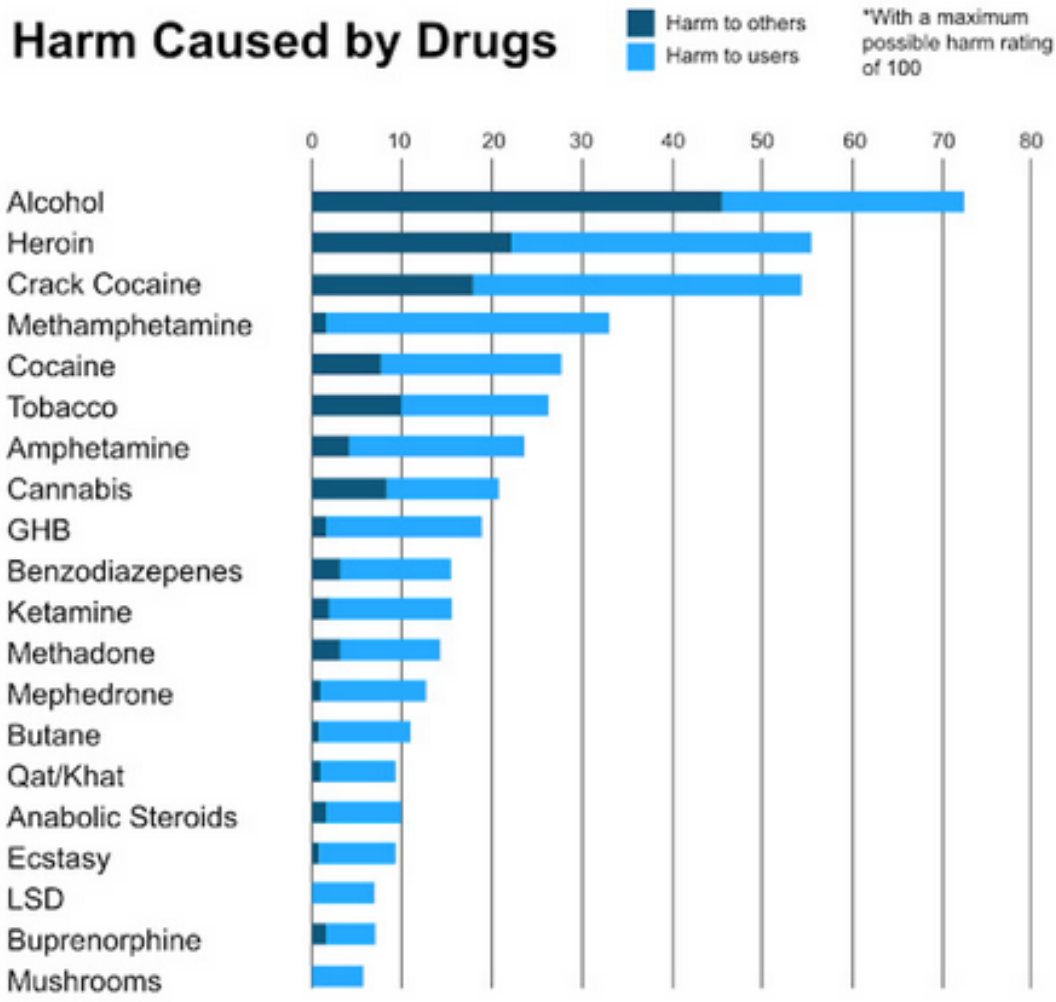


Figure 1-1 Harm caused by drugs in the UK.
(From Nutt et al, 2010)

Addiction

Addiction is a chronic neuropsychiatric and relapsing disorder, which causes changes in the reward pathway, that can lead to the overpowering motivational strength and decreased ability to control the desire to consume drugs (Koob *et al.*, 1998). There are several stages in the development of dependence. During the primary stages of the acquisition of addiction, it is widely accepted that rapid increases in dopamine in the nucleus accumbens is responsible for the subjective pleasurable effects of the drugs of abuse and natural rewards alike. As the behaviour becomes associated with the pleasurable effects, it is repeated in a Pavlovian manner. Later during the maintenance of a drug addiction there are marked decreases in dopamine function (Volkow *et al.*, 1997). It is thought that as the addiction progresses, the threshold

required for natural reinforcers to activate dopamine is increased (Martin-Soelch *et al.*, 2001). Cocaine addicts show altered brain response patterns to sexual stimuli using magnetic resonance imaging (MRI) (Garavan *et al.*, 2000) and smokers respond differently to non-smokers to monetary rewards (Martin-Sölch *et al.*, 2001). If addicts abstain from the drug of abuse they experience a number of negative side effects that become effective motivators themselves to maintain drug taking. Furthermore the drugs of abuse become more effective at blocking the negative reinforcement experienced during abstinence (Ahmed & Koob, 2005). Relapse is as high as 90% in the first year after abstinence and it is thought that non-pharmacological contextual factors, such as places, people or paraphernalia associated with drug intake, are important in craving and relapse (Robbins *et al.*, 2008). As these factors are repeatedly paired with pharmacological rewarding effects of the drug they are increasingly associated with the intense pleasurable experience, therefore they become powerful drug cues through Pavlovian conditioning alone (Robbins *et al.*, 2008). These cues play an important role in modulating the individual's expectations and behavioural responses to the drug. For example in drug addicts it has been shown that their attention and other cognitive and motivational processes are biased towards the drug and away from non-drug stimuli, which drives the strong motivational desire to consume the drug (Johanson *et al.*, 2006). Furthermore PET studies with [^{11}C]raclopride (dopamine antagonist) have revealed that videos showing cocaine paraphernalia can elicit a significant release in dopamine in the dorsal striatum, part of the 'reward' circuitry which is positively correlated with self reports of craving from cocaine addicts (Volkow *et al.*, 2006)**.**

Current treatments for drug addiction largely rely on substituting the drug of abuse with alternative source of reward to facilitate motivation for abstinence, such as methadone and buprenorphine for opioid abuse and nicotine patches, gum and breathalysers for nicotine addiction. These medications tend to be less potent and safer than the alternative. However due to the depression of the reward system these are often neglected (Jaffe, 1992) and it is necessary to continually provide escalating amount of the alternative (Higgins *et al.*, 2004). Rather than risking displacing the addiction to another substance there is increasing interest in treating the maladaptive learning processes that occur to help maintain abstinence.

Maladaptive learning and memory formation

Drug addiction can be viewed as a learned behaviour and molecular mechanisms similar to those underpinning cognitive learning and memory processes have also been implicated in drug addiction (White, 1996; Lu *et al.*, 2000; Hyman & Malenka, 2001; Kauer & Malenka, 2007). Different brain regions have been shown to contribute to the different aspects of addiction discussed above: acquisition, withdrawal, and relapse; and it appears that addiction involves multiple, complex adaptions that develop over the formation of each of these steps (for review see Kauer and Malenka, 2007).

Memory: the formation, consolidation and extinction of memories

In the 1950s Katz and Halstead hypothesised that the formation of a memory was dependent on a protein-based mechanism (Katz & Halstead, 1950). This idea wasn't tested until a decade later by Flexner et al (1965) who treated mice with puromycin, an antibiotic that inhibits brain protein synthesis, and showed an inhibition of memory formation (Flexner *et al.*, 1965). Since then this finding has been repeated countless times (Squire *et al.*, 1973; Davis & Squire, 1984; Nguyen & Kandel, 1996; Aguilar *et al.*, 2009). Throughout the 1970s experiments showed that periods of intense activity caused persistent increase in the strength of excitatory synapses. This lead to the hypothesis that alteration in synaptic strength in certain pathways or neuronal populations was responsible for the specificity of stored information. Squire and Barondes (1972) hypothesised that the proteins that were synthesised to produce neuronal changes were: 1) enzymes that controlled the synthesis and removal of neurotransmitters 2) postsynaptic receptor molecules 3) structural proteins 4) proteins that direct specialised types of intercellular recognition (Squire & Barondes, 1972). These ideas correlated with early work such as Hebb's (1949) hypothesis of activity-dependent changes in the strength of synaptic transmission and were later confirmed in hippocampal slices (Bliss & Lomo, 1973), and the concept of long term potentiation (LTP) was born. Largely these proposals have been substantiated experimentally leading to their general acceptance as the fundamental construct underlying initial memory formation. Animal experiments that have established a correlation between changes in synaptic strength and learning and memory have been

a turning point in understanding of how memory traces are encoded and stored in the central nervous system.

Traditionally, unlike short term (or working memory) it was thought that once established long term memory was stable (For review see Squire & Davis, 2003). However, it is now thought that new memories remain vulnerable to disruption after formation but progressively strengthen over time. The psychological literature introduced terms coined to explain several steps in memory formation. These were largely extrapolated from ethically controversial studies done in humans that showed that electroconvulsive shocks applied shortly after learning erased the memory (Duncan, 1949). Work done in animals since has confirmed that memory encoding involves two distinct steps: acquisition, which takes a few seconds followed by a series of changes that consolidate the new information to prevent disruption or decay, which can take as long as days. This new period of sensitivity has been confirmed by work done by Przybyslawski *et al.*, (1999) where memories were reactivated (recalled) and animals were then given a treatment known to disrupt consolidation during the original training. It is thought that two processes operate simultaneously when an animal enters the conditioned environment: the original association is retrieved and a novel association is made. In the reactivated state, both associations interact and the processing of the conditioned response may be disrupted (Aguilar *et al.*, 2009). This manifestation of memory that results in a behavioural change, such as recognition, constitutes a third component emerging immediately after acquisition but before consolidation occurs.

As expected, LTP also seems to show a progressive resistance to disruption, implicating it as a molecular mechanism for memory stabilisation. Immediately after induction of LTP *in vivo*, low frequency afferent stimulation easily extinguished the previous potentiation, but only in the first 30 minutes following treatment (Barrionuevo *et al.*, 1980). This finding has been repeated *in vitro* via transient hypoxia (Arai *et al.*, 1990) or a cooling paradigm (Bittar & Muller, 1993) and both completely eliminated LTP when applied within 5 minutes after the induction. Disruption of consolidation has also been shown in slice preparation with theta pattern afferent stimulation (Larson *et al.*, 1993) and becomes increasingly

inefficient over 30-minutes post induction (Huang *et al.*, 1999). These results suggest that reversal works by interrupting on-going synaptic processes that stabilises LTP. It is proposed that stages have different time frames depending on the time it takes to operate the cell machinery. For example adhesion proteins, such as the neural cell adhesion molecule (NCAM) have been shown to be correlated with the level of potential in hippocampal slices as soon as the potentiation occurs (Dityatev *et al.*, 2000). Later on however, protein and RNA synthesis is required for phases occurring more than an hour later. For example Nguyen & Kandel (1996) found that this formation of protein was dependent on cyclic adenosine monophosphate (cAMP)-mediated transcription within the hippocampus, and Long-LTP (a longer lasting form of LTP) was disrupted by inhibitors of cAMP-dependent protein kinase (PKA). The idea that the degree of initial encoding, and later consolidation, are dependent on the induction and stabilisation of LTP (For review see Lynch, 2002) is a fascinating one with interesting implications for drug discovery.

Animal models of reward learning

As there is no way to empirically measure learning, either behavioural changes (eg. CPP) or molecular changes (eg. LTP) are used as a correlate of learning. Most traditional models of learning and memory can be argued to have a motivational aspect to them. One of the earliest models, the radial arm maze (Olton *et al.*, 1977), relies on an animal's motivation to learn the position of a food reward in one of 8 arms of a maze. The model was developed to test both reference memory and working memory. Reference memory is assessed when rats remember and only visit the arms with the reward, whilst working memory is measured by number of times the animal visit the same arm in search for the reward. An adaptation of this model, the Morris water maze, is also a spatial navigational task that relies on the animal's ability to respond and recognise visual cues to locate a platform to escape swim stress. However these models have primarily been used to model spatial, working and reference memory and do not actually measure the motivation or the associative learning processes.

Associative learning is the process by which two stimuli or behavioural responses are learned to be linked or related to one another. The two forms of this type of learning

are operant and classical; the former relies on the principle of reinforcement of punishment, resulting in altered probability that the behaviour will occur again; whilst the latter relies on strengthening of repeated pairing of a previously neutral stimulus with an innately rewarding outcome. Most behavioural models of learning are a type of classical conditioning, where a conditioned stimulus (CS, an originally neutral stimuli), and an unconditioned stimulus (US, an innately rewarding stimulus) are presented sequentially, so the animal learns the association and consequently the probability of the behaviour is increased.

The main animal models of reward operate on the Pavlovian principle that a positive (rewarding) reinforcer once associated with a neutral stimulus will generate the elicited response to the neutral stimulus when it is presented alone. Two of the most widely used models of motivation and reward are based on these principles: conditioned place preference and self-administration.

Conditioned place preference (CPP) is an experimental protocol designed to model many types of behaviour including drug-seeking, reinforcement and motivational learning most commonly in rodents, but also zebra fish (Mustroph *et al.*, 2011), monkeys (Wang *et al.*, 2011) and even flat worms (Kusayama & Watanabe, 2000). The protocol consists of a neutral environmental cue (the conditioned stimulus, CS) becoming associated through classical conditioning with an unconditioned reward stimulus or motivational event, such as drug or food reward (Cunningham *et al.*, 2006). The motivational behaviour is measured by presenting the animal with a choice of environment, either containing the positive stimulus or the unbiased stimulus, post conditioning. If the unconditioned stimulus (US) is rewarding the animal is more likely to seek the environment associated with it (CS+). To date the general procedure as used by Rossi & Reid (1976) has been encompassed with some modifications by largely all subsequent CPP studies. Conditioned place preference can be thought of as a model of reward learning as it has been shown that learning and memory is required for reward (White, 1996; Lu *et al.*, 2000).

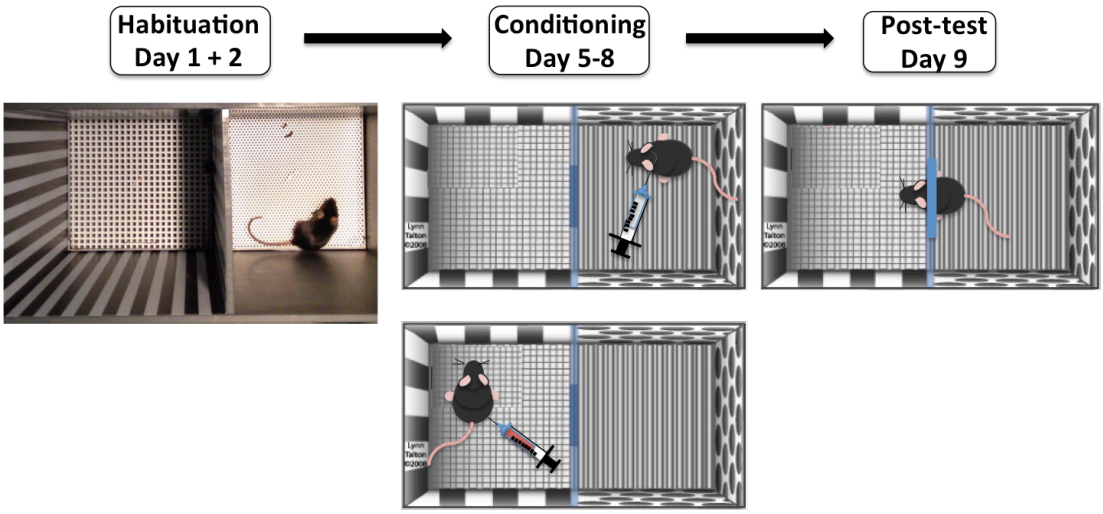


Figure 1-2 The general protocol for the acquisition of conditioned place preference.
During the habituation phase (Day 1-2) animals are allowed 2 x 15 minutes sessions to explore the boxes as the time spent in each context is recorded using Ethovision software. During days 5-8, animals are given daily alternating doses of either saline or drug dose in either side of the CPP box separated by the guillotine door. On day 9 animals are once again allowed to explore the whole apparatus in a drug free state and the time spent in each context is recorded.

In self-administration the animal performs a response, typically a lever press, which delivers a dose of drug via an intravenous catheter. Often, a light or tone, cue these responses, which become drug-associated cues. CPP and self-administration were once considered as equivalent measures of drug reward due to their ability to induce drug seeking behaviour with many of the same drugs (Bardo & Bevins, 2000). There is now much evidence that self-administration is a better model of human addiction as it is volitional and therefore has greater face validity. CPP on the other hand measures the relationship between a rewarding event and stimuli associated with that reward, which relies on the administration of drug by the experimenter. However, there are some criticisms of the self-administration model; due to the nature of the self-administration paradigm, drug is delivered often under the influence of drug and as the animal has little enrichment other than the lever for administration it may not be a true representation of drug seeking. Additionally repeated self-delivery of drugs are required to establish reliable drug seeking behaviour whereas a single CPP-trial has be shown to be sufficient (Mucha *et al.*, 1982). However, with CPP it can be difficult to interpret when animals prefer one environment prior to conditioning, and often there is huge variation in the response to conditioning across strains and even within the same strain and group of experimental animals (unpublished observation

from our laboratory group). The procedure has also been criticised for its lack of validity in modelling human drug reward, due to the absence of choice and social pressures associated with drug addiction. In the context of this thesis CPP has many advantages over self-administration. It tests animals in a drug free state reducing the likelihood of problems with sensitization and tolerance; it can be used to model learning of aversion as well as reward; it enhances understanding of the extinction and reinstatement of drug reward with small expense and relatively high throughput. Therefore the work presented in this thesis will utilise CPP as a model of reward learning, due to its speed and specificity for modelling particular aspects relevant to addictive behaviour. As with any behavioural work there are many parameters that need consideration before research studies begins. These limitations and parameters are considered and accounted for in appendix A.

1.2 The neurotransmitters of the mesocorticolimbic pathway and their role in reward learning

Dopamine

A huge development in the understanding of reward learning was the discovery of the mesocorticolimbic dopamine system. Dopamine (DA) is a multifaceted neurotransmitter that is involved in the fine tuning of motor function and cognitive function (Barron *et al.*, 2010), modulation of salience attribution and attention, as well as regulation of reward and motivation (Routtenberg & Lindy, 1965; Wise & Bozarth, 1987). Dopamine released from mechanosensory neurons in the nematode reduces crawling speed and causes the animal to take more turns (Hills *et al.*, 2004), in rodents it causes increased locomotion (Wise & Schwartz, 1981). This can be thought of as one of the simplest forms of reward seeking, as control of movement is critical for the animal in ensuring it spends as long as possible in the 'rewarding environment'. Dopamine was first associated with reward learning when it was found that stimulation of the dopaminergic brain region could be used to condition rats to lever press (Routtenberg & Lindy, 1965) and later dopamine antagonists were shown to reduce the motivation for a food reward before compromising the ability to make the response (Wise & Schwartz, 1981; Wise, 2004). All drugs of abuse have been shown to increase the release of dopamine in the nucleus accumbens via differential pharmacological effects (Di Chiara & Imperato, 1988).

The mesocorticolimbic dopaminergic pathway is composed of VTA dopaminergic neurons that project to the nucleus accumbens along the mesolimbic tract and the medial prefrontal cortex along the mesocortical tract. These midbrain dopamine cells are tonically active but show phasic activation following primary food rewards or stimuli associated with the presentation of a reward (Schultz, 2007). These regions are imperative to reward responding as 6-OHDA lesioning of these neurons (Smith et al, 1985), or the microinfusion of dopamine receptor antagonists into the NAc (Bachtell et al, 2005), or in knockout mice lacking dopamine D1 receptors (Caine *et al.*, 2007) all reduce drug reward seeking. These cells are thought to be important for prediction error. For example the switch from tonic to burst firing of dopamine cells is strongly enhanced by unexpected rewards far more than expected rewards, and the firing rate drops below baseline if a predicted reward never materialises (Schultz, 2001, 2007). This prediction of rewards is thought to be important for reward driven learning (Rescorla & Wagner, 1972; Schultz, 2000; Pessiglione *et al.*, 2006). The dopamine responses have also been shown to signal the value of the reward, for example lever presses coincided with peaks of dopamine in rats trained to press for sucrose rewards (Roitman *et al.*, 2004) whereas an aversive taste such as quinine supressed dopamine release (Roitman *et al.*, 2008).

Within this circuit dopamine has two main roles. The first is to inform the individual of the appearance of novel salient stimuli and thereby prompt neuromodulatory changes associated with learning and memory (Garris *et al*, 1999). Alternatively the spike of dopamine can prompt behavioural response previously paired with the stimuli. The two different tracts of the mesocorticolimbic circuit are thought to be primarily associated with these different functions. The mesolimbic circuit, projecting from the VTA to the nucleus accumbens, is primarily associated with motor behaviour and is thought to be critical in processing environmental stimuli and relaying this information to the motor circuit, which develops adaptive motor responses. Lesions of the mesolimbic fibres show impaired motor responses and impaired locomotor response to rewards (Jones & Robbins, 1992). The mesocortical pathway is thought to be primarily involved in cognitive control and motivational responses (Wise, 2004). The nigrostriatal dopamine fibres, that project from the substantia nigra (SN) to the basal ganglia and the striatum, are thought to be involved

in feeding and drinking behaviours as lesions cause deficits (Smith *et al.*, 1972) and also in addiction as stimulation of the SN in rewarding (Wise, 2009).

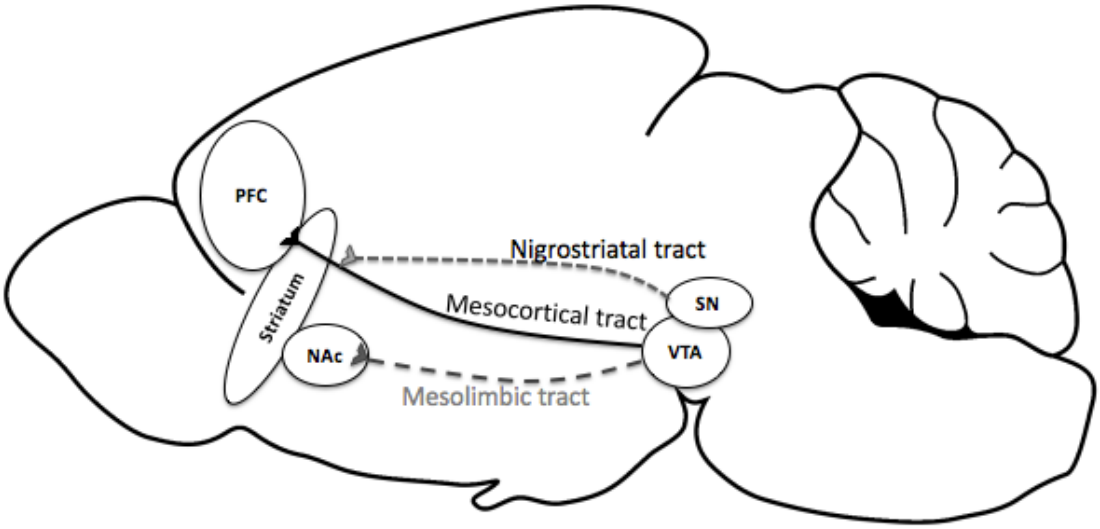


Figure 1-3 The main dopamine projections involved in mediating reward.
The mesocorticolimbic system arises in the ventral tegmental area (VTA) and comprises the mesolimbic projection to the nucleus accumbens, and the mesocortical projection to the prefrontal cortex. The nigrostriatal projection extends from the substantia nigra (SN) to the striatum.

While dopamine historically has claimed centre stage as the 'reward' transmitter, other neurotransmitters are recognised as playing crucial roles in the synaptic processes underpinning reward learning, notably the major excitatory and inhibitory transmitters in the mammalian CNS, glutamate and GABA.

Glutamate

Throughout the 20th century the hypothesis was developed that the brain underwent long lasting, activity-driven changes that enabled it to translate and store memories that can last for years and even decades, as discussed above in section 1.1. However it was only in the late 1960's that empirical evidence emerged that showed the repetitive activation of excitatory synapses in the hippocampus caused an increase in synaptic strength that could last for hours, and even days (Hebb, 1949; Bliss & Lomo, 1973). Research over the last decade has suggested an important role for glutamate in modulating these responses (Blackstone *et al.*, 1992; Sucher *et al.*, 1996; Ozawa, 1998; Borges & Dingledine, 1998; Tzschentke & Schmidt, 2003; Guo *et al.*, 2009; Ferrario *et al.*, 2010; Caffino *et al.*, 2014).

Glutamate acts through glutamate receptors (GluRs) that are divided into two distinct groups, ionotropic and metabotropic receptors. The ionotropic receptors (iGluRs) are further subdivided into three groups defined by their specific agonists, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropioate (AMPA), Kainate and N-methyl-D-aspartate (NMDA) receptor channels. These receptors are either homo-oligomeric, or hetero-oligomeric structures assembled from distinct subunits to form cation-selective tetramers (AMPA, GluA1-4, NMDA GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A and GluN3B; Kainate, GluK1-5). The metabotropic receptors (mGluRs group 1-3), however, are coupled to GTP-binding proteins and regulate the production of intracellular messengers.

Both AMPA and NMDA receptors have been implicated in long-term potentiation (LTP) the change in strength of a synapse. There are many types of synaptic plasticity, but the most studied is NMDAR-dependent long-term potentiation, which was first observed in the hippocampus (Bliss & Collingridge, 1993). It requires coincident presynaptic glutamate release and postsynaptic depolarization, the former to activate NMDAR and the latter to relieve the voltage-dependent block of the NMDAR by Mg^{2+}. NMDAR opening allows the influx of Ca^{2+}, which is a crucial trigger for LTP, by activating intracellular signaling cascades such as those involving CaMKII (Malenka, 1999). In addition Ca^{2+} influx through calcium permeable channels and transitions of G-protein linked receptors can activate signalling molecules within these cascades. The convergence of multiple signalling molecules leads to cAMP-responsive element-binding protein (CREB) activation which suggests that this transcription factor plays a critical role in integrating different inputs and mediating appropriate long term neuronal responses (Carlezon et al, 2005). For example known targets of CREB include genes for neuropeptide Y (Wand, 2005), dynorphin (Cole et al, 1995), brain derived neurotrophic factor (BDNF) (Nibuya et al, 1995)**,** as well as the AMPA subunit, GluR1 (Olson *et al.*, 2005). As discussed previously protein synthesis and transcription of these genes has shown to be essential for the formation of memories. For example activation of ERK/MAPK is required for the formation of spatial memories (Sweatt, 2001) and perhaps as a consequence acquisition of amphetamine-induced place preference is

inhibited by the administration of ERK and p38 MAP kinase antagonists (Gerdjikov et al, 2004).

Most researchers believe that ultimately the enhanced postsynaptic currents that define LTP are caused by changes in AMPA receptors (AMPAR) to a greater extent than NMDA receptors (NMDAR) (Nicoll *et al.*, 1988; Perkel & Nicoll, 1993), but the exact mechanism for this is more widely debated. Two hypotheses stand; either more receptors are added to the synapse to increase the response to a given amount of neurotransmitter; or extant receptors are modified to enhance their operation (Lee *et al.*, 2000). AMPARs are anchored to the postsynaptic density (PSD) via scaffolding proteins and cytoskeletal elements (Lisman & Raghavachari, 2006; Okabe, 2007; Newpher & Ehlers, 2008). It is thought that synaptic AMPARs are recruited either from intracellular vesicle stores or extrasynaptic surface sites via lateral diffusion through a process known as AMPA trafficking. Furthermore the GluR subtypes differ considerably in their permeability to calcium, GluR2 containing receptors are Ca^{2+} impermeable, therefore making this subtype a key determinant of AMPAR function (for review see Cull-Candy *et al.*, 2006). It has been shown that subunit switching occurs within synapses in an activity dependent manner, for example there is an increase in receptors lacking GluR2 subunits after morphine administration (Billa *et al.*, 2010). This process is highly dynamic and due to its importance in controlling synaptic strength is highly regulated. Even at baseline states AMPARs are thought to be undergo continuous exchange from synaptic sites to different store pools to allow steady state levels at synapses (Choquet & Triller, 2003; Shepherd & Huganir, 2007; Newpher & Ehlers, 2008; Triller & Choquet, 2008). Synaptic activity can dramatically change this balance and either prompts recruitment or removal of synaptic AMPARs. These processes all require accessory molecules that are thought to control exocytosis of the intracellular pool of AMPARs, lateral diffusion to synaptic sites and retention at synapses via scaffold interactions (Opazo & Choquet, 2011). For example over expression of the TARP member Stargazin as well as PDZ-containing scaffolding proteins present in the PSD (Kim & Sheng, 2004) strongly mobilises AMPARs to the surface (Schnell *et al.*, 2002). Protein kinase A (PKA) activation strongly increase exocytosis of AMPARs by direct phosphorylation of AMPAR (Man *et al.*, 2007) and the molecular motor

myosin V is essential for LTP expression as it mobilised the AMPAR containing vesicles to the cell surface (Wang *et al.*, 2008).

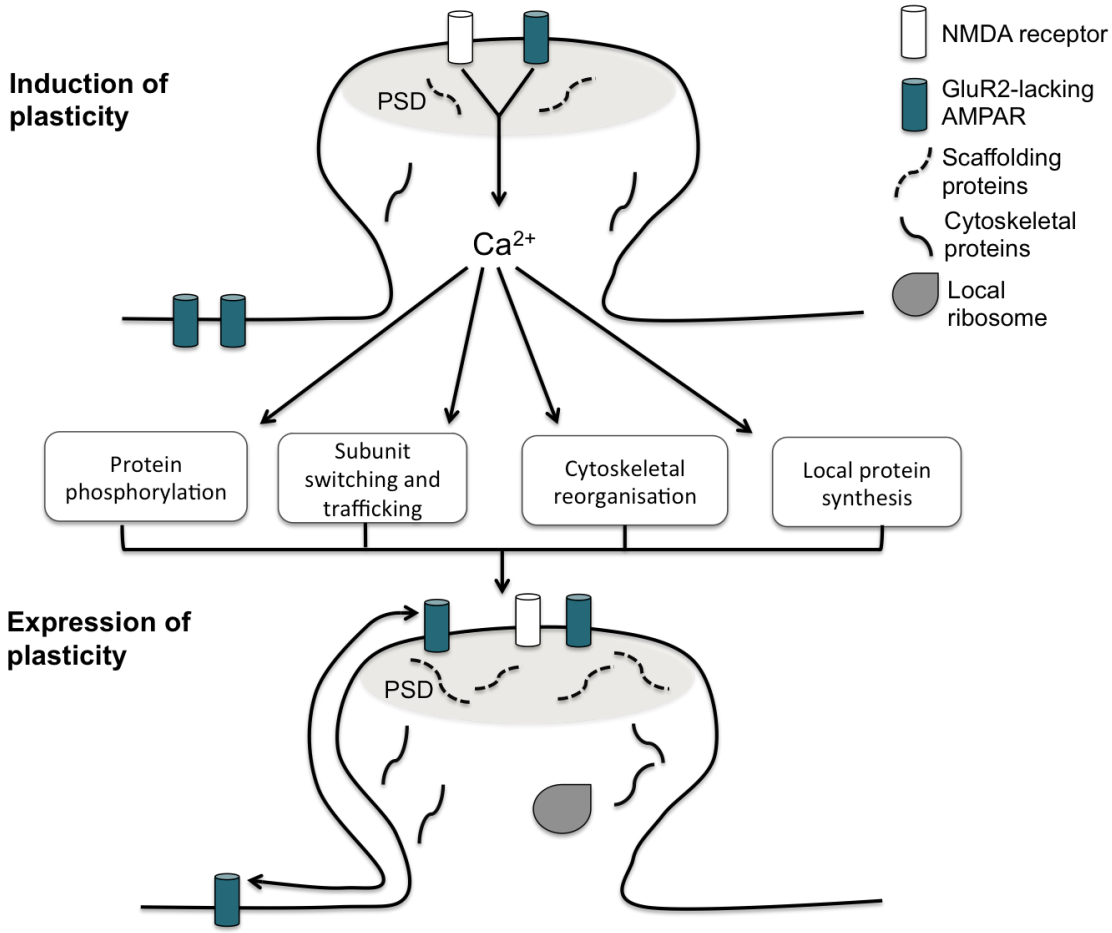


Figure 1-4 The mechanism for controlling glutamate-dependent synaptic plasticity.
Ca^{2+} through GluR2-lacking AMPAR and NMDAR trigger signalling cascades that control the phosphorylation of proteins involved in trafficking, cytoskeletal organisation and protein synthesis. Either LTD or LTP can occur at these excitatory synapse through AMPAR trafficking from extrasynaptic and synaptic sites (arrow), up-regulation of scaffolding and cytoskeletal proteins to anchor the receptors in the PSD. Adapted from (Derkach *et al.*, 2007).

GABA

GABAergic signalling is also thought to be essential in modulating motivational responses. The main projection neurons of the NAc and dorsal striatum are GABAergic and it is thought that there is a decrease in GABAergic output from the NAc shell after acute drug use (Nestler, 2001). Furthermore VTA DA cells (Kalivas *et al.*, 1990) and glutamatergic neurons in the hippocampus (Paulsen & Moser, 1998) are modulated by GABAergic neurons. It is thought that GABA regulates overall

circuit tone and thereby serves to bind or sustain an animal's motivational state until the goal object can be achieved (McFarland *et al.*, 2003)**.** The importance of these GABAergic projections has been emphasised, particularly in the genesis of rhythmic activity in the medial septum and hippocampus, known as theta rhythm. GABAergic signalling has been shown to be important in controlling relapse to heroin, as an increase in the inhibitory GABAergic synaptic inputs received by the mPFC pyramidal cells occurs after re-exposure to heroin–conditioned cues (Van den Oever *et al.*, 2010).

Can the reward circuitry be influenced by ACh signalling?

Acetylcholine (ACh) is a less abundant transmitter compared to glutamate or dopamine, but it is released throughout the mammalian nervous system where it has been shown to impact on sleep wake cycles, attention, memory formation and more recently reward. Compared to its fast actions at the neuromuscular junction and autonomic ganglia, in the CNS ACh largely mediates neuronal excitability, alters presynaptic release of neurotransmitters, and coordinates firing groups of neurons**.** Experimental evidence is emerging that suggests that rising levels of acetylcholine may play an important role in reward reinforcement. Levels of intra-accumbal ACh have been shown to increase after morphine (Crespo *et al.*, 2006) cocaine (Williams & Adinoff, 2009), ethanol (Larsson *et al.*, 2005) but not sucrose self-administration (Crespo *et al*, 2006). Furthermore cholinergic interneurons in the striatum are thought to be critical for mediating the association between drugs of abuse and the environmental cues that drive drug taking and relapse after drug cessation (Exley & Cragg, 2008).

Cholinergic inputs of the brain

In the 1980s the antibody for the ACh synthesising enzyme was generated and its use revealed the intense cholinergic innervation of the rodent brain. A schematic of the cholinergic projections in the rodent brain is shown in figure 1.5 The main cholinergic system sends afferents from the basal forebrain and is formed from four main structures. The medial septum (MS), the vertical limb of the diagonal band of Broca (VDB) which projects via the substantia innominate (SI), and the nucleus basalis of Meynert (NBM). The VDB extends to the SI which forms the main

projection to forebrain structures while the NBM sends massive projections to neocortical structures. The brainstem cholinergic neurons included the lateral dorsal tegmental (LDT) and pedunculopontine tegmental nuclei (PPT) which project to the hind brain, thalamus, hypothalamus, and basal forebrain (Paul *et al.*, 2015)**.** These projections also innervate the substantia nigra and the ventral tegmental area (Woolf & Butcher, 1986; Hallanger & Wainer, 1988; Lavoie & Parent, 1994). Cholinergic action within this region has been shown to increase DA activity and GABAergic signalling, that prompts burst firing and phasically increased efflux in terminal regions (Nisell *et al.*, 1994; Corrigall *et al.*, 1994; Westerink *et al.*, 1996; Forster & Blaha, 2000).

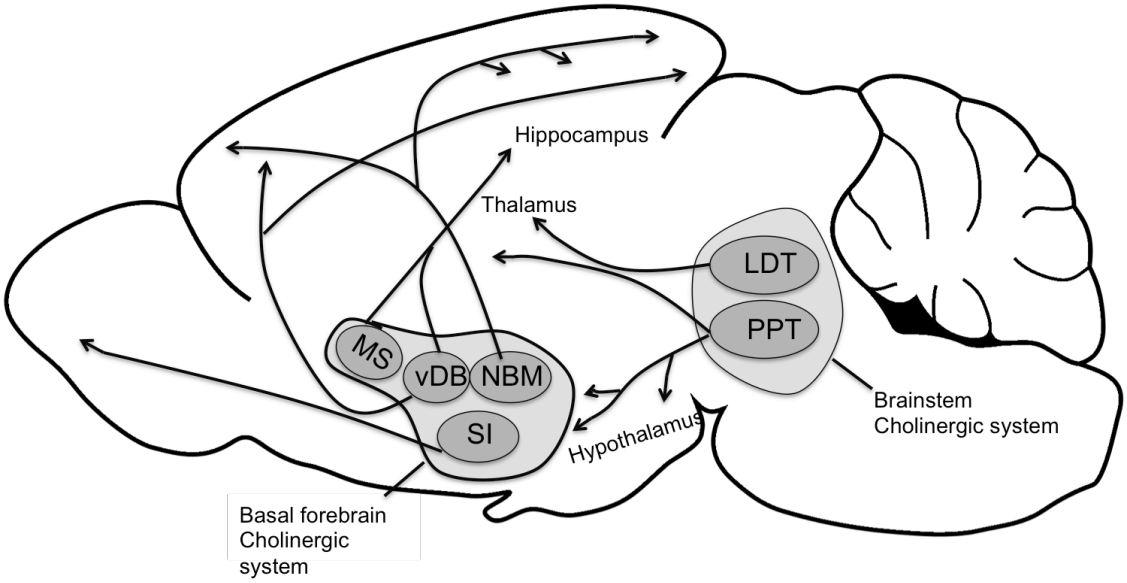


Figure 1-5 The main cholinergic projections in the brain.
The basal forebrain projections included the medial septum (MS), ventrical limbs of the diagonal band of broca (vDBB), nucleus basalis of Meynert (NBM), and substaintia innominate (SI) which project to the hippocampus, thalamus and cortical regions. The brainstem cholinergic system includes the lateral dorsal tegmental (LDT) and pedunculopontine tegmental nuclei (PPT) that project to the hypothalamus and thalamus (Paul et al., 2015).

The septo-hippocampal (SH) pathway, which arises from the medial septal nucleus (MSN) and the nucleus of the diagonal band, is the main source of cholinergic input to the hippocampus. This has been investigated intensively and a topographical model of the innervation across the septo-temporal axis has been constructed based on various lesion (Mellgren & Srebro, 1973; Pearson *et al.*, 1987), tracing and immunocytochemistry studies (Crutcher *et al.*, 1981; Chandler & Crutcher, 1983; Nyakas *et al.*, 1987). The septal neuron innervates the hippocampus via three main

projections: the fimbria, the dorsal fornix, and the supracallosal striae. A fourth route via the amygdala complex which terminates mainly in the subiculum has also been described. The pathway is topographically organised along the mediolateral and rostrocaudal axes with laterally located neurons projecting more ventrally into the hippocampus (Amaral & Kurz, 1985).

Within the hippocampus the septal fibres terminate in a laminar pattern in the dentate gyrus and CA1 regions. Cholinergic fibres are relatively evenly distributed in the layers of the hippocampal CA1 except for two bands of high density in the stratum pyamidale (SP) and at the border between the stratum radiatum and stratum lacunosum moleculare (Nyakas *et al.*, 1987; Aznavour *et al.*, 2002). These projections are different in the ventral and dorsal hippocampus. The CA1 pyramidal and dentate granule cell layers in the dorsal hippocampus receive afferent inputs from the vDBB, whereas cells in the ventral hippocampus receive inputs from both the vDBB and MS (Nyakas et al, 1987).

Until recently it was thought that the striatum contained only a few GABAergic interneurons compared to the large diversity seen in the hippocampus (Freund & Buzsáki, 1998) however, it is now thought to also contain ACh interneurons. In the CNS the striatum is the region most densely occupied by ACh interneurons, these are large aspiny, slow tonically firing cells (Kawaguchi *et al.*, 1995). In primates these cells undergo a burst-pause pattern of firing during motor learning and reward behaviours (Aosaki *et al.*, 1994). Furthermore it is thought that this pattern of firing causes tonic low level DA release when the interneurons are firing, and a reduction in tonic DA, but the interneurons maintain phasic release when they pause (Exley & Cragg, 2008).

1.3 Nicotinic acetylcholine receptors

Due to the diverse function and location of the receptors for ACh, as well as the surge of research implicating them in learning and memory, there is great interest in investigating the role they have in modulating reward learning. This thesis aims to

outline the role they have in modulating reward behaviours with conditioned place preference (CPP).

ACh is known to exert its effects on two distinct receptor types: muscarinic receptors (M1-M5), and nicotinic receptors. Muscarinic receptors (mAChRs) belong to the family of G-protein-coupled receptors, which mediate slow metabolic responses to ACh. The focus of this thesis is on nicotinic receptors (nAChRs), which are less numerous than the mAChRs in the CNS and their roles are predominantly modulatory (Dajas-Bailador & Wonnacott, 2004) although some examples of nAChRs mediating synaptic transmission exist (for example in the VTA). nAChRs also mediate the effect of ACh in muscle, autonomic ganglia and some sensory organs (Jensen *et al.*, 2005). More recently evidence suggests nAChRs are also present on non-neuronal cell types such as lymphocytes, glia, macrophages, dendritic cells, adipocytes, keratinocytes, endothelial cells, human platelets (Schedel *et al.*, 2011) and epithelial cells of the intestine and lungs (Sharma & Vijayaraghavan, 2002). They are commonly located on presynaptic terminals where they have been shown to modulate neurotransmitter release independently of depolarisation. In particular they have been shown to modulate the release of glutamate and dopamine (Livingstone *et al.*, 2010) and therefore been implicated in the modulation and control of memory, learning and reward behaviours.

Structure and classification of nAChRs

Neuronal and muscle type nAChRs are similarly structured to other members of the 'cys-loop' family of ligand-gated ion channels, such as $GABA_A$, 5-hydroxytryptamine receptors ($5\text{-}HT_3$) and glycine receptors (Le Novère & Changeux, 1995; Miller & Smart, 2010)**.** Understanding of the receptors' structure was hugely advanced by the purification and study of muscle type nAChRs in the marine ray *Torpedo marmorata's* electroplax. The abundance and accessibility of this receptor in the electroplax of such electrogenic animals has made it an excellent model for understanding the structure and function of all 'cys-loop' ligand gated ion channels (Changeux *et al.*, 1970). The arrival of molecular cloning techniques allowed detailed study of the subunits and to date 17 different nAChRs have been cloned, 5 of which are expressed in muscle (α1, β1, δ, γ, ε) and 12 nAChRs in the CNS (α2-10,

β2-β4) (Patrick *et al.*, 1989; Miller & Smart, 2010; Fasoli & Gotti, 2015, summarised in table 1.1)**.**

Each subunit consists of an external N-terminus, which plays a role in ligand binding, several highly conserved membrane spanning helices (M1-4), which form the ion conduction path (Gotti et al, 2006) and play a role in the conformational change upon activation (Miyazawa et al, 2003) and a large cytoplasmic loop between M3 and M4 (Figure 1.6). In the CNS the α and β subfamilies are categorised based on the presence or absence of a conserved pair of vicinal cysteine residues only present on α-type, and critical for agonist binding (Karlin, 1993). Co-expression of these different subtypes into oocytes of xenopus laevis and electrophysiological studies revealed only a small number of nAChR subunit combinations were functional in terms of receptor formation and activation of ionic currents on application of nAChR ligands (table 1.1). These subunits form pentameric transmembrane proteins surrounding a cation-permeable pore (Ca^{2+} and $Na^{+)}$, each comprised of different subunit combinations, that have different pharmacological properties (Fenster et al, 1997).

The availability of the α-bungarotoxin specific ligand has allowed the characterisation of two distinct types of nAChRs: the α-Btg-insensitive including all α/β heteromeric subtypes and the α-Btg-sensitive which include both the homomeric and heteromeric (in the brain predominantly α-only) types (Nai et al, 2003). α-BGT sensitive receptors are typically made up of only α7 subunits in the rodent (Cui *et al.*, 2003) but may be homoeric α7 or α8 or α7-α8 receptors in the chick (Gotti *et al.*, 1994). However there is some evidence that the α7 subunit can form functional heteromeric receptors with β2 (Liu *et al.*, 2009)**.** This primarily homomeric structure of α7 nAChR is responsible for the unique functional properties that allow them to mediate cellular events involved in learning and memory and consequently are the focus of this thesis.

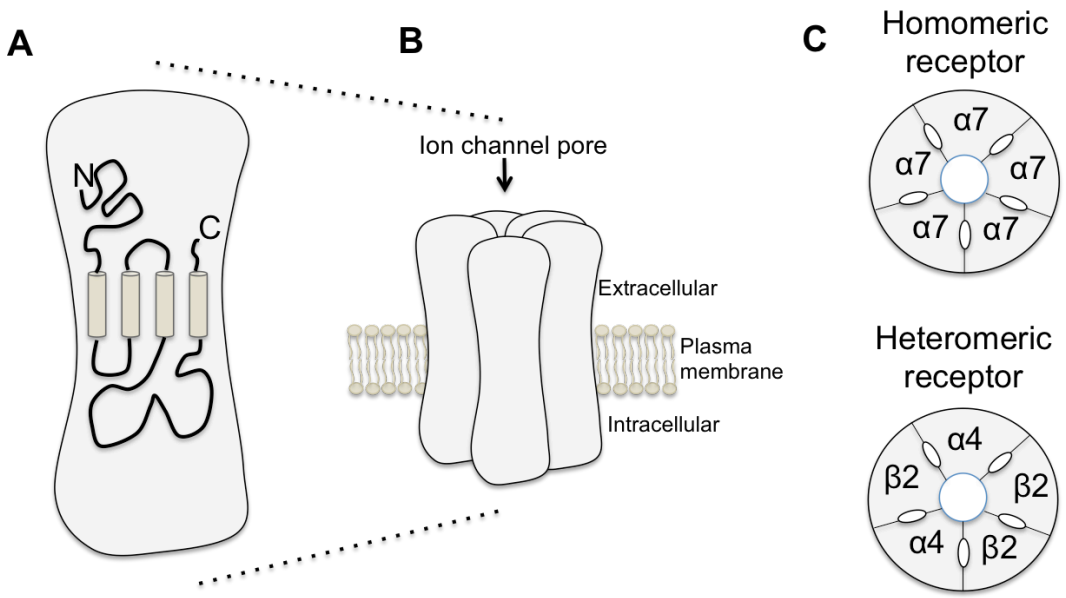


Figure 1-6 The Structure of the nAChR.
A) Schematic diagram of the structure of nAChR subunit, showing the N and C terminus, the membrane spanning regions and the variable cytoplasmic loop between M3 and M4. B) The organisation of the subunits to form a functional receptor. C) Subunit arrangement is specific to each subtype. (Gotti & Clementi, 2004).

Table 1-1 The potential physiological subunit combinations of the nAChRs and where they were first characterised.
Data collected from expression studies coupled with electrophysiology has revealed two main groups: muscle type and neuronal type. nAChRs present in sensory epithelia do not fall into either category. (Millar & Gotti, 2009).

Receptor Subtype	Subunits	Subtypes and where they were first charaterised
Muscle-type	α1, β1, δ, ε, γ	**α1, β1, δ, γ** (Embryonic muscle) **α1, β1, δ, ε,** (Adult muscle)
Neuronal, α-BGT in sensitive	α2-α6, β2-β4	**α2 β2** (Xenopus oocyte) **α2 β4** (Xenopus oocyte) **α3 β2** (Xenopus oocyte) **α3 β4** (Xenopus oocyte) **α4 β2** (Xenopus oocyte) **α4 β4** (Xenopus oocyte) **α6 β2** (HEK cells) **α6 β4** (Xenopus oocyte, Chick retina) **α 2α5 β2** (Chick optic lobe) **α3 α5 β2** (Xenopus oocyte) **α3 α5 β4** (Xenopus oocyte) **α6 β3 β4** (Xenopus oocyte) **α3α5β2β4** (Xenopus oocyte) **α3α5 β2β4** (Chick ciliary ganglion) **α3α6β3β4** (Xenopus oocyte, Chick ciliary ganglion) **α4α5α6β2** (rat VTA and substantia nigra neurons) **α4β2β3β4** (COS cells)
Neuronal, α-BGT sensitive	α7, α8	**α7** (Xenopus oocyte) **α8** (Xenopus oocyte, Chick optical lobe) **α7β2** (Xenopus oocyte, rat forebrain) **α7β3** (Xenopus oocyte) **α7α8** (Chick retina, Xenopus oocyte)
Other	α9, α10	**α9** (rat cochlear hair cells, Xenopus oocyte) **α9α10** (rat cochlear hair cells, Xenopus oocyte)

Cellular localisation

nAChR location and the type of neuron that they occupy determine the specialised properties and functions of the receptors (Dajas-Bailador & Wonnacott, 2004; Albuquerque *et al.*, 2009). Most anatomical and functional evidence suggest that they are primarily located at presynaptic (Wonnacott, 1997)**,** and pre-terminal sites, where they can regulate neurotransmitter release in several parts of the brain (McGehee *et al.*, 1995). The presence of the α7 nAChR at neuronal presynaptic sites was demonstrated in hippocampal and olfactory bulb slice preparations continuously perfused with tetrodoxin. Under these conditions a nicotinic agonist was able to increase the frequency of miniature excitatory postsynaptic currents in an α-BGT sensitive manner (Alkondon *et al.*, 1996, 1998). The similarity to nAChRs at the neuromuscular junction led to the expectation that nAChRs would be also be found on postsynaptic neurons. A few lines of evidence exist that suggest synaptic transmission can be mediated by nAChRs (Zhang *et al.*, 1993, 1996a; Roerig *et al.*, 1997; Ullian *et al.*, 1997; Hefft *et al.*, 1999) but on the whole excitatory nicotinic transmission by ACh acting on nAChRs has been difficult to document due to the varicose nature of cholinergic projections.

Location within the rodent brain

The whereabouts of nicotinic receptors in the rodent brain (the model used throughout this thesis) has been well characterised from data obtained from binding and immunocytochemistry (Figure 1.7**)** (Fuchs, 1989; Séguéla *et al.*, 1993; Hill *et al.*, 1993; Hills *et al.*, 2004)**.** Much more recently the development of subtype-specific compounds and knockout mice has allowed for the precise mapping of receptor subunit localisation within different brain areas (Zoli *et al.*, 2002). The β2 subunit is expressed in almost all areas of the CNS and α4, although less abundant, is co-localised with β2 in most regions forming the most highly expressed receptor α4β2* nAChR (Wada *et al.*, 1989) now known to be capable of including additional subunits not cloned in 1989. The dopaminergic neurons projecting to the striatum have been well documented to have four populations: α4β2, α4α5β2, α6β2β3, and α4α6β2β3 (Champtiaux *et al.*, 2002; Zoli *et al.*, 2002; Salminen *et al.*, 2004)**.** β2 subunits have highest expression in the cortex (particularly layers III and IV) hippocampus and thalamus (Whiting & Lindstrom, 1987; Wada *et al.*, 1988; Hill *et*

al., 1993)**.** The focus of this thesis, the α7 nAChRs, have been found in abundance in the cortex (layers I and VI) and the hippocampus (particularly high in the CA3), amygdala, inferior colliculus, and the VTA (Fuchs, 1989; Séguéla *et al.*, 1993) but not in the thalamus or striatum (Sargent, 1993). It has recently been demonstrated that α7 and β2 subunits are co-expressed in the rat basal forebrain cholinergic neurons, to form α7β2 subtype that has different pharmacological properties to the homomeric type (Moretti *et al.*, 2014). The expression of nAChRs within the reward system is shown in figure 1.8.

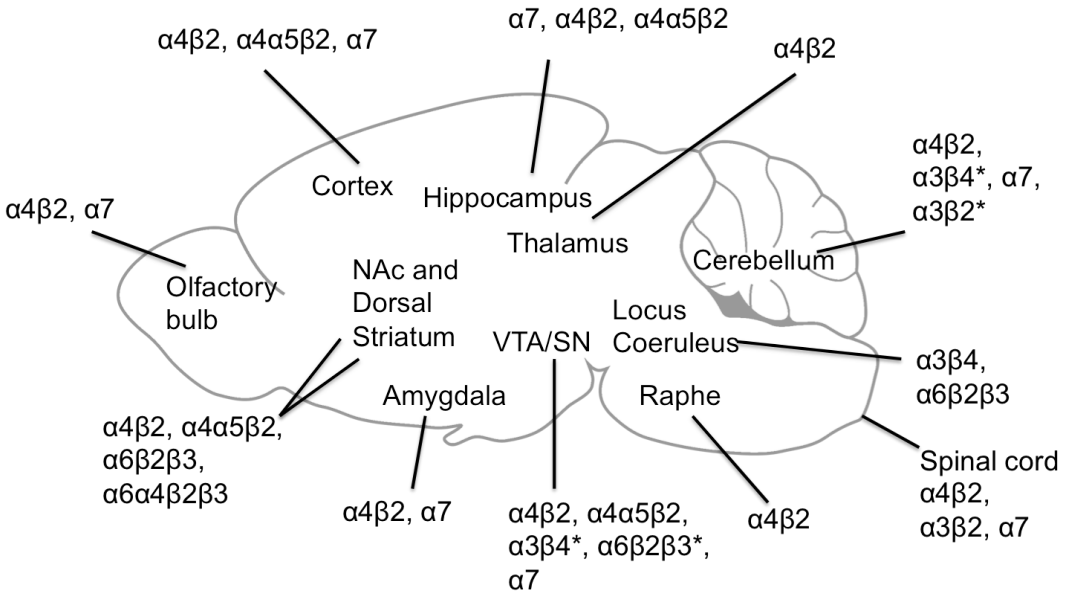


Figure 1-7 Distribution of nAChR subtypes in the rodent brain determined by biochemical, pharmacological and functional studies.
The mesolimbic dopamine pathway originating in the ventral tegmental area (VTA to the nucleus accumbens (NAc) and the nigrostriatal pathway from the substantia nigra (SN) to the dorsal Striatum are shown. (*) demonstrates subtypes with yet unconfirmed co-assembly. Modified from (Wonnacott *et al.*, 2005; Millar & Gotti, 2009)

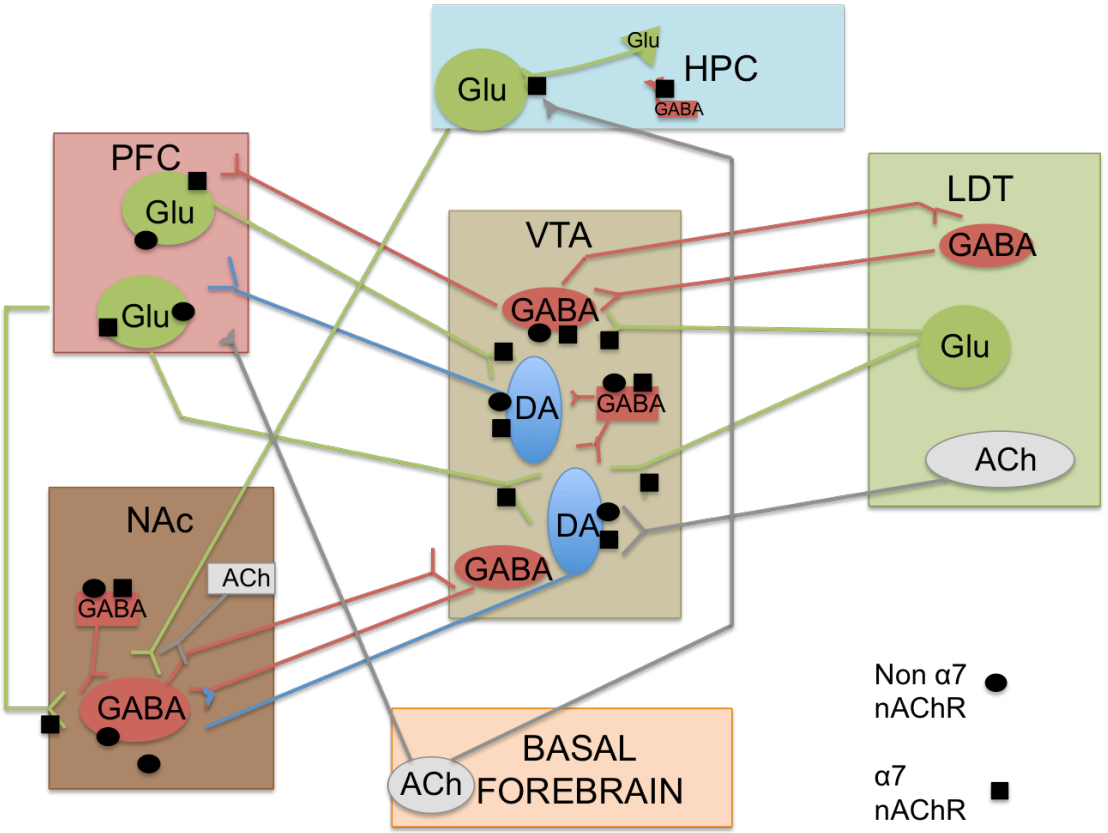


Figure 1-8 A summary of the main excitatory and inhibitory projections of the reward pathway.
The projections of the ventral tegmental area (VTA) to the prefrontal cortex (PFC), nucleus accumbens (NAc). Cholinergic input from the basal forebrain to the hippocampus (HPC) and PFC, and the lateral dorsal tegmentum (LDT) to the VTA. Non α7 are represented as circles and α7 nAChRs are represented as squares. Interneurons are denoted by the square boxes. (Wonnacott, et al, 2005; Millar & Gotti, 2009)

Functional properties as ion channels

The nAChR is thought to have three main functions as an ion channel: it moderates the energy barrier for ionic conductance, it selects among ions and exists in several different function states (Karlin, 2002). The different structures within the channel contribute to these functions, particularly the M2 segment of each subunit (Unwin, 1995)**.** The selectivity of the channel is abolished if this region is mutated as is its ability to undergo conformational change (Imoto *et al.*, 1988; Leonard *et al.*, 1988)**.** New research techniques have allowed the relative permeability of the nAChRs subtypes to be well characterised. Traditionally ionic reversal of potential shifts were determined to estimate the receptor's permeability to Ca^{2+} and Na^{+}, however fluorescence-based methods are now used which give much more reliable estimates. As discussed above it is thought that the subunit composition of the nAChR has a significant impact on its relative permeability to Ca^{2+}. For example heteromeric receptors consisting of α- and β- subunits appear to be less permeable to Ca^{2+} as they have a fractional Ca^{2+} current of only 2-5% (Fucile *et al.*, 2005). The homomeric structure of the α7 however, makes this receptor the most permeable to Ca^{2+}, possessing a fractional current range from 6-12%.

The receptors can exist in one of four distinct states: resting (R), active and two closed channel states, intermediate (I) and desensitised (D) (Changeux *et al.*, 1984; Quick & Lester, 2002; Albuquerque *et al.*, 2009)**.** The rate constants between these functional states are dependent on the specific combination of subunits and the chemical characteristics of the ligand that is bound. In particular the α7 receptor undergoes a very rapid desensitisation with relatively fast recovery. Consequently peak currents cannot be used to determine the response of a particular agonist, and α7 receptors are often referred to as low affinity receptors for ACh. However when the concentration-response functions are corrected to incorporate the rapid desensitisation, the EC_{50} of ACh is roughly equivalent to that of the putative high affinity receptors (Papke *et al*, 2002). Consequently, the α7 nAChRs, due to its tendency to rapidly desensitise, and the α4β2 nAChR for its high affinity for ACh, ultimately mean that in an environment of diffuse ACh they are likely to be desensitised. This has important implications for their physiological roles within the brain.

Physiological roles of nAChRs in the brain

As discussed much is known about the structure and expression of nAChRs but this knowledge surpasses what is known about their role in modulating CNS function. The ability of nAChRs to modulate biological function relies on their ability to translate the binding of an agonist, such as ACh, to receptor transition, which allows the influx of ion flow and ultimately a cellular response.

Regulation of neurotransmitter release

Due to the predominant expression on presynaptic and pre-terminal neurons nAChRs have been shown to directly and indirectly modulate neurotransmitter release (MacDermott et al, 1999; Wonnacott, 1997). In particular presynaptic nAChRs have been implicated in the release of ACh (Wilkie *et al.*, 1993) noradrenaline (NA) (Clarke & Reuben, 1996), DA (Rapier *et al.*, 1990; Grady *et al.*, 1992), glutamate (McGehee & Role, 1995; Alkondon *et al.*, 1997; Dickinson *et al.*, 2008) and GABA (Yang *et al.*, 1996). Microdialysis studies have shown that systemic administration or direct application of nicotine into the VTA or NAc (Pontieri et al, 1996), results in dopamine overflow in the NAc. Dopamine release is controlled by the firing pattern of the mesolimbic neurons, and binding of the agonists to a nAChR triggers the switch from single-spike firing to burst firing therefore more efficiently raising DA levels. Furthermore, since nAChRs located in the VTA are tonically active due to cholinergic afferents from the lateral dorsal tegmental nucleus they can modulate both dopaminergic as well as glutamatergic transmission under basal conditions (Champtiaux et al, 2006).

Mediating long-term cellular events

There is evidence that nAChRs are present on excitatory synapses postsynaptically and that they can mediate fast synaptic transmission in the CNS (Zhang *et al.*, 1993, 1996; Roerig *et al.*, 1997; Ullian *et al.*, 1997; Hefft *et al.*, 1999). By increasing glutamate presynaptically and increasing depolarisation postsynaptically nAChR can induced rapid changes in membrane potential allowing the transduction of events at the synapse to the nucleus triggering long-lasting changes. A unique feature of all synapses is their ability to undergo activity-dependent changes in synaptic strength, a function termed synaptic plasticity (Kauer & Malenka, 2007). Of particular

importance to this function is the receptor's permeability to Ca^{2+} in regulating activity, survival, and fate of neurones. Initially it was found that nicotine could, acutely and chronically, facilitate the induction of long term potentiation (LTP) (Fujii *et al.*, 1999). Nicotine, when added in a brain slice preparation, causes long lasting LTP that is inhibited by mecamylamine. Both nicotine and choline when given *in vivo* induce similar long lasting potentiation in the dentate gyrus of the hippocampus (Matsuyama *et al.*, 2000). DMXB, a novel nicotinic agonist, facilitated the induction of LTP in the hippocampus in a dose dependent and mecamylamine sensitive manner (Hunter *et al.*, 1994). Furthermore the nootropic agent, nefiracetam, induces an 'LTP-like facilitation' that could be blocked by either alpha-bugratoxin or mecamylamine, or a selective protein kinase C inhibitor (Nishizaki *et al.*, 1999).

Central to this role is the ion channel's permeability to Ca^{2+}, an important signalling messenger with broad actions within neurons. The homomeric structure of the α7 however, makes this receptor the most permeable to Ca^{2+}, possessing a fractional current range from 6-12%. As well as Ca^{2+} influx through the channel itself, nAChRs are capable of activating Ca^{2+} release from intracellular stores. It has been shown that *in vitro* activation of Ca^{2+} stores following stimulation of nAChRs contributes to long-lasting Ca^{2+} signals (Dajas-Bailador *et al.*, 2002b). This influx of Ca^{2+} can result in the activation of specific signalling cascades implicated in learning and memory (Figure 1.9). It has been shown that nAChRs mediate the Ca^{2+}-dependent activation of ERK/MAPK, CAM -kinases and CREB in several neuronal models (Nakayama *et al.*, 2001; Chang & Berg, 2001; Dajas-Bailador *et al.*, 2002b; Hu, 2002).

The likelihood of desensitisation of the receptors implicated in cognition, α7 and α4β2, pose an interesting question. In diffuse ACh these receptors are likely to be desensitised due to the high affinity for the agonist and high low affinity open state in the later. Desensitised nAChRs have been reported to potentiate certain forms of synaptic plasticity, and in the rat hippocampus methyllylaconitine (MLA), an α7 selective antagonist (Davies et al, 1999), mimicked the nicotine induced potentiation of LTP (Fujii et al, 2000). The role of desensitised receptors is still under debate, but it seems probable that nAChRs under this state are unlikely to modulate

neurotransmitter release or increase cell excitability via activation of the postsynaptic membrane. Therefore, the receptors baseline physiological role may be to prevent over-excitation in response to increase cholinergic signalling, thereby selectively filtering and relaying information to higher modalities of the CNS. As the endogenous concentration of ACh is unknown it may be that these receptors change from their desensitised state with a relatively small change in ACh.

At first glance the varying effects of nAChR agonism and antagonism on plastic events may seem confusing. More recent evidence has suggested that the effect of nAChRs may be dependent on the timing of synaptic events (Ji *et al.*, 2001b; McGehee, 2002a; Yakel, 2012). For example the timing of input of activation from the septal cholinergic fibres projecting to the hippocampus can induce different forms of plasticity that depend solely on the timing of the input (Yakel, 2012). When activated it can induce hippocampal plasticity with a timing precision in the millisecond range. For example when the input to the CA1 was activated 100ms prior to activation of the Schaffer collateral (SC) pathway, this induced an α7-dependent LTP that required the activation of NMDA receptors and the insertion of GluR2-containing AMPA receptors in the spines. However if activated only 10ms prior to the SC pathway this induced a α7 dependent short-term depression (STD) that was mediated primarily through the presynaptic inhibition of glutamate release (Gu & Yakel, 2011).

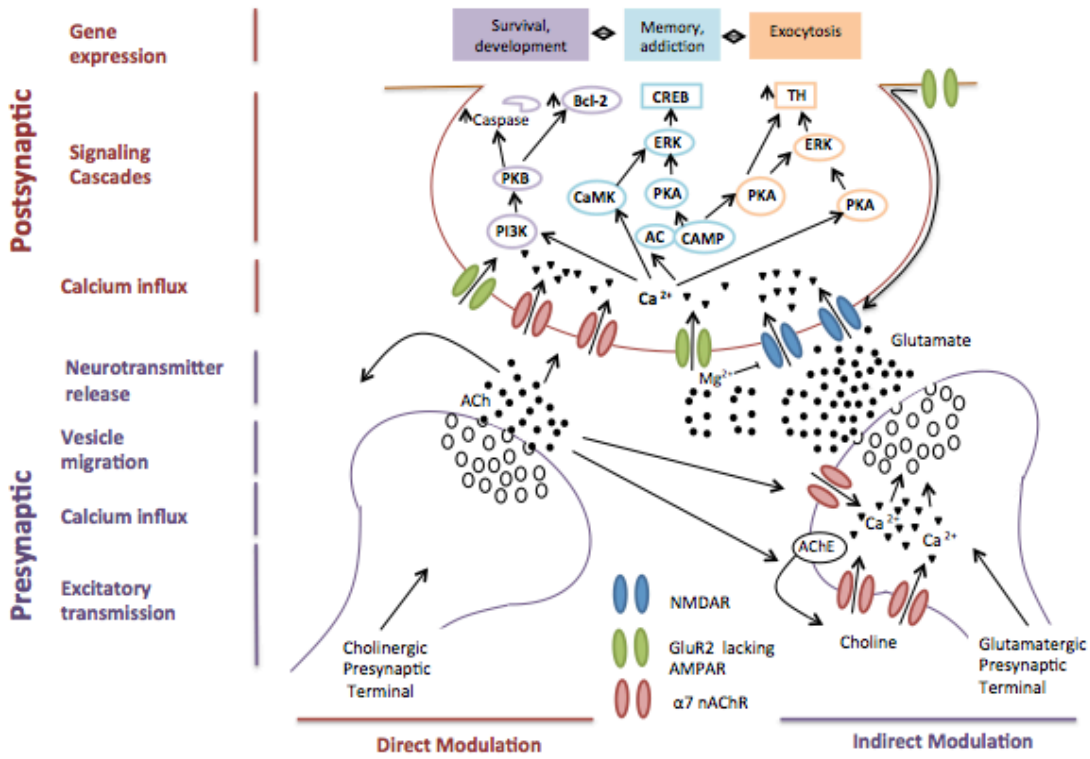


Figure 1-9 Schematic Diagram depicting the hypothetical roles for alpha7 nAChRs in the CNS. Presynaptic α7 nAChRs can modulate the release of neurotransmitter when present on presynaptic terminals. When activated by ACh, nAChRs undergo a conformational change which allows the influx of ions causing membrane depolarisation and the migration of neurotransmitter containing vesicles to the presynaptic membrane. There is some evidence of direct modulation of the postsynaptic neuron by nAChRs (Zhang *et al.*, 1993, 1996; Roerig *et al.*, 1997; Hefft *et al.*, 1999) through acetylcholine release from cholinergic neurons; but indirect modulation through α7 induced glutamate from glutamatergic neurons is well excepted. On interaction with the ligand post-synaptic α7 nAChRs and NMDA receptors can regulate ion influx which further increase membrane depolarisation lifting the inhibition of mg2+ and consequently further increasing permeability to ca2+. The high intracellular concentration if calcium activates adenylyl cyclase (AC), protein kinase A (PKA), PKC, ca-calmodulin-dependent protein kinase (CaMK) and phosphatidylinositol 3-kinase (PI3K). Figure adapted from (Broide & Leslie, 1999; Dajas-Bailador & Wonnacott, 2004).

1.4 Preclinical and clinical evidence implicating nAChR in mediating learning and memory

Endogenous acetylcholine release by cholinergic neurons may be necessary to modulate acquisition, consolidation, reconsolidation, extinction and expression of memories. Not surprisingly, because of their role in modulating synaptic plasticity, nAChRs have been implicated in studies of learning and memory (Levin, 2013). Nicotine's effect on cognition is well reported in healthy humans (Decker *et al.*, 1995; Brioni *et al.*, 1997; Levin, 2002) and in people with illnesses such as Alzheimer's disease and in schizophrenic adults with attention deficit hyperactivity disorder (Jones & Robbins, 1992; Le Houezec *et al.*, 1994; Levin *et al.*, 1996a,b). In particular the α7 nAChRs have been proposed to represent a therapeutic target for treating a number of CNS disorders, including schizophrenia and Alzheimer's (Lindstrom, 1997). Due to the lack of selectivity of these naturally occurring ligands there has been great interest in developing new compounds with selective effects on different nAChR subtypes both for therapeutics but also as a research tool to advance understanding of the nAChRs. Significant effort has been invested in α7 selective compound discovery and this has yielded many α7 agonists and partial agonists. Drugs that enhance cholinergic transmission have been identified as promising targets for the treatment of cognitive impairments in the elderly, as well as schizophrenia (shown in table 1.2).

Table 1.2 New α7 compounds trialled in the clinic.
The preclinical data for these compounds largely support their efficacy in treating a range of cognitive deficits including attention, working and recognition memory (Kitagawa *et al.*, 2003)

Compound	Type	Literature
GTS-21	Agonist	(Arendash *et al.*, 1995; Briggs *et al.*, 1997; Meyer *et al.*, 1997; Woodruff-Pak, 2003)
AR-R17779		(Levin *et al.*, 1999; Mullen *et al.*, 2000; Van Kampen *et al.*, 2004
ABBF		(Biton *et al.*, 2007; Pichat *et al.*, 2007; Hashimoto *et al.*, 2008)
PHA-709829		(Buccafusco *et al.*, 2007; Tietje *et al.*, 2008
SENI12333/WAY-317538		Feuerbach *et al.*, 2007
PNU-282987		Boess et al., 2007
SSR180711	Partial agonist	Lopez-Hernandez et al., 2007; Lagostena et al., 2008; Marighetto et al., 2008
A-582941		Acker *et al.*, 2008
JN403		(Rezvani *et al.*, 2009)
S 24795		Roncarati *et al.*, 2009
MEM3454		Hajós *et al.*, 2005
PNU-120596	Positive modulator	Hurst *et al.*, 2005
NS1738		Timmermann et al., 2007
CCMI		Ng et al., 2007)
TQS		Grønlien et al., 2007
SB-206553		Dunlop et al., 2009

Animal *in vivo* experimental data also shows support for an involvement of nAChRs in learning and memory. Chronic and acute nicotine administration has been shown to facilitate working memory (Levin & Simon, 1998; Rezvani & Levin, 2001). Whilst hippocampal infusions of mecamylamine, a potent competitive non-selective antagonist, were found to impair working memory but not reference memory in rats (Ohno *et al.*, 1993). Vicens *et al* (2011) showed that PNU-282987 (1mg/kg), an α7 selective agonist, diminished the acquisition of the Morris maze task in rodents These findings are consistent with other studies at the same dose, for example PNU-282987 has been found to restore amphetamine-induced auditory sensory gating deficiency (Hajós *et al.*, 2005). If the α7 nAChR agonist, choline, is infused into the hippocampus immediately after training, expression of the memory is increased, and vice versa if methyllylaconitine (MLA), an α7 selective antagonist, is infused (Blake *et al.*, 2014). Hippocampal infusions of nAChR antagonists impair, whilst agonists enhanced short and long-term memory in rats (Martí Barros *et al.*, 2004). Particular attention has been paid to the roles of the α7 and α4β2, as MLA and Dihydro-β-erythroidine (DHβE), an antagonist with specific binding to α4β2 at the sub-micromolar range (Gotti *et al.*, 2006a) both cause a significant increase in errors made in working and reference memory (Levin, 2002). Furthermore, MLA given immediately after memory reactivation, impaired reconsolidation in mice trained with either a mild or high foot shock (Boccia *et al.*, 2010).

Reward

Tobacco use and dependence on other drugs of abuse show high co-morbidity, for example smoking prevalence in ethanol-dependent individuals is approximately three times higher than in the general population (Istvan & Matarazzo, 1984; Sobell *et al.*, 1990) and ethanol consumption is higher in smokers than non-smokers (Shiffman & Balabanis, 1992). Furthermore early dependence on nicotine is associated with increased risk of addiction to other drugs later in life (Loimer *et al.*, 1991). The idea that memories are liable to change during reconsolidation offers an opportunity to pharmacologically weaken the maladaptive memory structures that support relapse in drug addicts.

Animal models of reward

Acetylcholine has been implicated in modulating reward, key evidence suggest that VTA acetylcholine levels rise during eating and drinking (Rada *et al.*, 2000) and inactivation of the PPT, the primary input of acetylcholine into striatal areas, has been shown to impair conditioned reinforcement (Inglis *et al.*, 2000) as well the ability of VTA DA neurons to switch to burst firing (Pan *et al.*, 2005). There appears to be a dichotomy between the effect of mAChRs and nAChRs in modulating rewarding behaviours. Activation of both muscarinic and nicotinic acetylcholine receptors in the accumbens by ACh volume transmission was necessary for drug conditioning but only muscarinic receptors seem to be important in reward responding for food (Yeomans *et al.*, 1993; Crespo *et al.*, 2006; Sharf & Ranaldi, 2006). Specifically repeated infusions of scopolamine but not mecamylamine, prevent rats from acquiring operant behaviour for food delivery (Sharf & Ranaldi, 2006).

The drug for which nAChRs play an obvious role is nicotine, the major psychoactive and addictive compound in tobacco, where they act as the primary site of action in mediating craving and withdrawal. Like many other drugs of abuse, nicotine enhances mesocorticolimbic dopamine transmission DA increases in the NAc (Pontieri *et al.*, 1996). The nAChRs in the VTA and the NAc are thought to be imperative to the rewarding properties of nicotine (Corrigall *et al.*, 1992, 1994), in particular the α4β2 nAChR is thought to be important as infusion of (DHβE) into the VTA decreases nicotine self-administration (Corrigall *et al*, 1994). Therefore several FDA and European approved smoking cessation agents act as either partial agonists or antagonists at the nAChR. Cysteine, a natural product from *Cytisus laborinum* that is marketed in Europe as Tabex® and its analog Varenicline developed by Pfizer (Chantix®), act as partial agonists at α4β2-containing nAChRs and have been tested in models of smoking cessation. Levin *et al* (2010) has shown that Sazetidine-A, a novel desensitising agent and partial agonist with high α4β2 selectivity, reduces nicotine self-administration in preclinical models.

In animal studies antagonists such as mecamylamine have also been shown to reduce cue-induced reinstatement of nicotine seeking in rats (Liu *et al.*, 2007). This effect on

acquisition, maintenance and relapse of nicotine addiction (Biala *et al.*, 2010) is thought to be mediated through α4β2* and α3β2* subtypes. However, the α6 selective antagonist, α-conotoxin MII (α-CtxMII) blocks nicotine-stimulated DA release in rat striatal synaptosomes (Kulak *et al.*, 1997) and preclinical studies have shown its potential in reducing nicotine self-administration (Crooks *et al.*, 2014). Galantamine, an acetylcholineterase (AChE) inhibitor and positive allosteric modulator at α7 nAChRs has been shown to reduce both nicotine self-administration and reinstatement of nicotine seeking behaviour (Hopkins *et al.*, 2012). Furthermore, it has been found that intra-VTA administration of methyllycacontitine (MLA), the selective antagonist for α7 nAChR (Ward *et al.*, 1990), attenuates the rewarding effects of nicotine (Laviolette & van der Kooy, 2003).

nAChRs can also modulate responses to other drug s of abuse

Nicotinic antagonists inhibit mesolimbic DA release induced by a number of psycho-stimulant drugs (Zanetti, 2006). Clearly the neurochemical evidence would suggest an important role for nAChRs in drug reward. An aim of this thesis (see section 1.5) is to evaluate any contribution of nAChRs, specifically α7 nAChRs, in mediating the response to drugs that do not primarily target the cholinergic system. Therefore this introduction focuses on α7 nAChR, with comparative information about other nAChR subtypes.

The mechanism by which alcohol elevates DA release is largely unclear (Okamoto *et al.*, 2006; Dopico & Lovinger, 2009). Although alcohol is not a direct agonist it has been hypothesised that it induces increase in ACh firing in the LTD resulting in elevated levels of ACh in the VTA which could potentially drive DA increases (Larsson *et al.*, 2005). Non-selective antagonism of nAChRs has been shown to reduce ethanol seeking for example both systemic or local administration of mecamylamine (VTA: Ericson *et al.*, 2008), reduces ethanol seeking (Ericson *et al.*, 1998; Lê *et al.*, 2000; Söderpalm *et al.*, 2000). It has been suggested that this effect is largely dependent on the α6β2* receptors as dihydro-β-erythroidine (DHβE) failed to supress ethanol consumption (Larsson *et al.*, 2002). However, varenicline, another selective α4β2* receptor antagonist, selectively decreases ethanol consumption and seeking (Steensland *et al.*, 2007) although it is known to target other subtypes in

addition to those that it was developed for (Reus *et al.*, 2007). Furthermore varenicline and cysteine (Sajja & Rahman, 2013) have been shown to reduced cue-induced alcohol relapse (Wouda *et al.*, 2011; Sajja & Rahman, 2013). α7 nAChR blockade, with MLA, has been shown to be ineffective in reducing ethanol consumption (Kamens *et al.*, 2010).

Furthermore an effect of α4β2 nAChRs has been seen in methamphetamine (Verrico *et al.*, 2014), and cocaine reward in rodents (Guillem & Peoples, 2010) but is ineffective in reducing cocaine self-administration in primates (Gould *et al.*, 2011). The nicotinic antagonist mecamylamine (1mg/kg) disrupted place preference to cocaine and mice lacking the β2 subunit showed decreased cocaine preference (Levin *et al.*, 2000; Zachariou *et al.*, 2001; Champtiaux *et al.*, 2006). Finally MLA, an α7 antagonist was found to reduce cocaine reward (Panagis *et al.*, 2000).

Preclinical evidence suggests that nAChRs are important modulators of drug reward and specific receptors seem to have different effects on different psycho-stimulants. Research suggests a role for both α4β2 and α7 in mediating opiate reward. Feng *et al* (2011) found that pre-treatment with either MLA or DHβE 20 minutes prior to the administration of morphine priming dose, inhibited reinstatement of morphine-CPP (discussed in detail in Chapter 3), however evidence is limited and it is as yet unclear at which stages of the reward learning nAChRs are implicated. Due to their role in modulating neurotransmitter release and neuronal plasticity, α7 nAChR are the thought to be involved in the drug associated learning that is hypothesised to be important in mediating relapse.

1.5 Aims of Thesis

There is a growing body of evidence that implicates the nAChRs in modulating responses to rewarding stimuli (Feng *et al*, 2011; Rezayof, 2006). The evidence for nAChRs, notably α7 nAChRs, having a role in modulating responses to non-nicotinic drugs of abuse leads to the hypothesis that inhibition of α7 nAChRs would diminish drug-induced responses. The aim of this thesis is to exploit the selective antagonism of α7 nAChRs to explore their involvement in mediating different stages of the rewarding process elicited by a non-nicotinic drug, namely morphine.

During the first part of this work a model of reward learning, **Conditioned Place Preference (CPP), was optimised and validated** (Appendix A). In the light of evidence that suggests that memory encoding undergoes a number of stages in which the memory remains malleable, the paradigm was designed to explore these different stages of memory formation: the acquisition, expression, reconsolidation and reinstatement of a context dependent drug reward. This model was utilised in Chapter 3, with the aim to explore **the effect of inhibition of nAChR using the nAChR antagonists**, MLA, an α7 specific antagonist, and mecamylamine, a non-selective antagonist with low affinity for α7, in each of these 4 different stages of context-dependent drug reward learning.

The location of nAChRs throughout the circuitry implicated in motivational learning puts them in a prime location to mediate synaptic plasticity (Fujii & Sumikawa, 2001; Ji *et al.*, 2001b; McGehee, 2002b; Cobb & Davies, 2005; Maylie & Adelman, 2010), so chapter 4 aimed **to detect changes in AMPA and NMDA receptors after morphine reinstatement and to see if these are affected by pre-treatment with MLA.** To do this [^{3}H]MK-801 and [^{3}H]AMPA binding was monitored throughout the brain, using quantitative autoradiography, following reinstatement of morphine-CPP, with and without MLA pre-treatment.

Finally, in chapter 5, **to investigate the locus of action of nAChR modulation of CPP**, intracranial delivery of MLA to candidate regions implicated by the autoradiography data in the previous chapter was conducted. As intracerebral administration requires implantation of an in-dwelling cannula, it was necessary to carry out this study in rats; therefore, earlier effects of MLA were extended to male Wistar rats.

CHAPTER 2 MATERIALS AND METHODOLOGY

2.1 Statistical analysis

All data are presented as mean ±standard error of the mean (S.E.M). The conditioned place preference data is presented as time spent in drug-paired side -450s (half of the post-test time) to give a preference score that represents the increase in time spent on the drug-paired side. Exclusions were made if the time spent in either side exceeded 10 minutes during habituation (2% occurrence) or if in reinstatement experiments the animals failed to display acquisition of morphine-CPP (~8%). All behavioural analysis was done in *in vivo stat* with a one-way ANOVA with repeated measures, *post hoc* analysis with Benjamini-Hochberg test for multiple comparisons. The MLA intracranial delivery analysis was conducted as a multiple t-test. The autoradiography data were analysed with a 2-way ANOVA with an effect of pre-treatment (MLA) and treatment (morphine).

2.2 Behavioural Protocols

Animal housing and care

All experiments were performed in accordance with Home Office project licence held under 'ASPA' 1986 and approved by a local ethical review panel. Male C57B6/J mice, 6 weeks old were obtained from Charles River, UK; whilst all male Wistar rats (400-450g) were sourced from University of Bath breeding colony. All animals were housed in groups of four, except animals that underwent surgery which were housed singly post operatively, in a behavioural holding room with controlled temperature (24±2 °C), humidity (50-60%), and a 12:12h light-dark cycle (lights on: 0600-1800). Food and water was available *ad libitum*. Mice and rats were allowed to adapt to laboratory conditions for at least 1 week before the procedure during which they were handled daily in the experimental room. All experiments were carried out in the light phase and 6-26 animals were used in each experimental treatment. Weekly cage cleaning was conducted by the experimenter, immediately after behaviour on post-test days, allowing two days of recovery before the next stage of the protocol.

Drugs

Morphine hydrochloride was purchased from MacFarlan Smith, Edinburgh, UK; methyllycaconite (MLA) was purchased from Tocris Cookson, Bristol, UK. PNU-

120596 and PNU-282 987 was a generous gift from Pfizer. Mecamylamine was purchased from Tocris Cookson, Bristol, UK. Morphine was dissolved in sterile saline at 1mg/ml and injected at a volume of 10 ml/kg intraperitoneally (mouse: i.p) or subcutaneously (rat: s.c.). Control animals received saline injections (sodium chloride 0.9% w/v, Hameln pharmaceuticals, Gloucester, UK) in the same volume and by the same route of delivery.

CPP Procedures

A series of initial experiments were performed to optimise the CPP procedure (see Appendix A). The whole procedure consisted of eight different experimental stages: Habituation trial (1x15 minute session/day for 2 days), Conditioning (1x40 minute trial/day for 4 days), Post-conditioning test (1x15 minutes trial), then either Reconsolidation (40 minute session in the drug paired side) or Extinction (1x30 minute trial/day for 4 days), or Maintenance or Expression test (1x15 minutes trial/per day as required) and Reinstatement (1x30 minute trial). The whole protocol and which steps were used for each experiment is shown in figure 2.1. Data was collected via a camera and analysed through a PC equipped with an auto-monitoring system (Ethovision XT version 8.0). Different protocols were selected to investigate different aspects of the CPP protocol (see figure 2.1).

Apparatus

Mouse: The apparatus (UGO Basile, cat no. 42503) consisted of a two-compartment box (16cmx15cm each), one black and white striped and the other plain black, separated by removable guillotine doors. The floors of each chamber differed, either having round 2mm holes or 4x4mm square holes respectively (see appendix A). The conditioning apparatus was contained within a custom-made sound attenuation chamber (MED Associates, UK).

Rat: The apparatus (MED Associates, UK) consisted of a two-compartment box (30cmx30cm each), one vertical and one horizontal black and white striped, separated by removable guillotine doors and a neutral zone measuring (10x30cm). The floors of each chamber differed, either having round 2cm holes or 1x1cm square holes respectively.

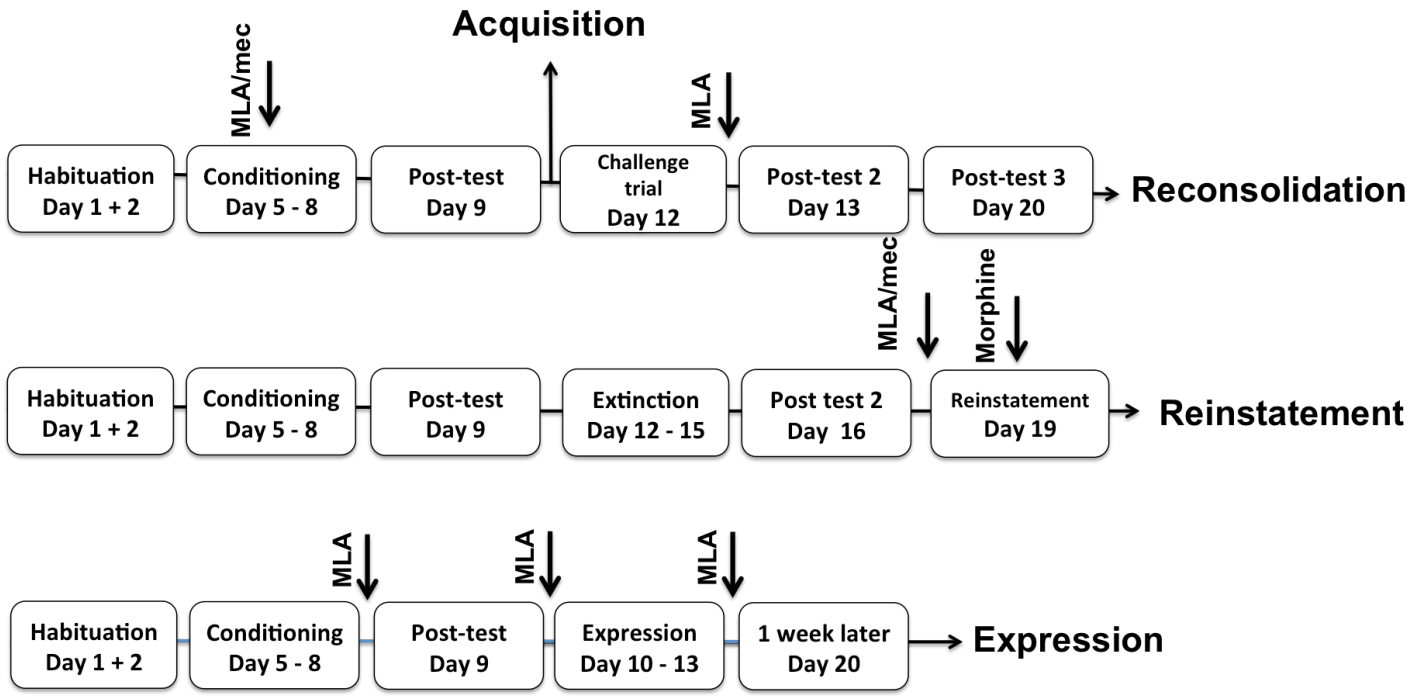


Figure 2-1 The protocol for all conditioned place preference (CPP) behavioural experiments.
Protocols for acquisition, reconsolidation, reinstatement and expression are shown. Drug treatments are shown as arrows. All protocols were used to investigate the effect of methyllycaconitine (MLA), but only acquisition and reinstatement were tested with mecamylamine (MEC).

Habituation test

On the first day of the study each mouse was placed in one of the compartments and allowed access to the CPP box without the guillotine door for 15 minutes. The software measured time spent (s) in either side as well as locomotor activity (distance moved, cm) and this was used to determine any initial preference or aversion to either compartment. This was repeated in a second habituation test (see appendix A). These data were then used to assign experimental treatments in a pseudo-randomised way to create a counterbalanced design. For example, half of the animals were morphine conditioned to the black side and half to the striped side, and the order in which the animals received morphine was counterbalanced in a similar way.

Conditioning

Two days after the habituation test (day 5), animals were injected with either morphine or saline and confined to either side of the apparatus. The following day animals that received morphine on day one were injected with saline and confined in the opposite side to day one. This was repeated again for day 3 and 4 so each animal received 4 conditioning trials (2x saline, 2x morphine). The treatment compartment was assigned according to a counterbalanced design, such that one half of the animals in each experimental group were conditioned with morphine to the side nearest the laboratory door and the other half the side furthest away. The order of presentation of morphine was also counterbalanced, half of the animals received morphine day 1, whilst the others received saline. On day 2 this was reversed, so that drug naïve animals received their first morphine dose, and so on for a total of 4 conditioning days. After each 40 minute trial, animals were returned to their home cage in the holding room.

MLA pre-treatments: After habituation animals were pseudo-randomly allocated to one of four treatment groups. Group A: received saline (10ml/kg, s.c.) 20minutes prior to the conditioning dose of either morphine (10mg/kg, i.p) or saline (10ml/kg, i.p); group B: received MLA (4mg/kg, s.c.) followed by a priming dose of morphine (5mg/kg, i.p). Group C: received saline (10ml/kg) 20minutes prior to saline on all days. Group D: received MLA (4mg/kg) 20 minutes prior to saline (10ml/kg) on all days. All pre-treatments and conditioning doses were administered daily in the

experimental room and in between the pre-treatment and treatment animals were returned to their home cages.

Post-conditioning test (Post test)

On day 9, 24 hours after the last morphine treatment animals were placed in the CPP box with the guillotine doors removed to allow access to both chambers for 15 minutes. Time spent in each compartment and total distanced moved (cm) were recorded using Ethovision.

For experiments where the effects of MLA on expression, reconsolidation and reinstatement of morphine-CPP were tested, all animals first underwent acquisition of morphine-CPP as shown above, without treatment with MLA during the acquisition phase.

Expression

In experiments to test expression, or maintenance, of previously acquired morphine-CPP, animals were trained to acquire morphine CPP (up to experimental day 8, see figure 2.1). Then on day 9 animals received MLA (4mg/kg, s.c) or saline control, 20 minutes prior to a 15 minutes preference test with the guillotine doors removed to allow access to both chambers. The same cohort of animals then underwent further post-test session on day 10-13 and day 20 (MLA or saline injections 20 minutes prior to each 15 minute preference test). Time spent in each compartment, the distanced moved (cm) was recorded using Ethovision.

Reconsolidation

In experiments to test reconsolidation of morphine-CPP, animals were trained up to day 9 (see figure 2.1). On day 12 all animals received a morphine dose (10mg/kg, i.p) and were immediately placed into the drug paired side with the guillotine door closed for a challenge trial. Immediately after the 40-minute session animals were given MLA (4mg/kg, s.c) or saline before being return to their home cage. 24 hours and one week later animals were placed into the CPP box with the doors open and tested for 15 minutes for their preference.

Extinction

To test the effect of MLA or mecamylamine on the reinstatement of morphine-CPP, animals underwent acquisition of morphine-CPP up to day 9, then underwent extinction training. From day 12 animals received daily extinction training for 4 days. The procedure followed the same method as the conditioning phase except all animals received saline in both chambers. On day 16 animals under went a second post-test (post-test 2) and only animals meeting the criterion (no more than 70% of time spent in the drug-paired chamber, less than 5% occurrence) were used in subsequent phases (reinstatement).

Reinstatement

On reinstatement day (day 19) animals were randomly allocated to one of two treatment groups. 12 animals received a saline (10ml/kg,,i.p) or morphine priming dose (5mg/kg, i.p.) prior to the trial. Preliminary experiments revealed the second 15 minutes of the reinstatement trial showed higher reinstatement of morphine-CPP, and these data were used for later analysis.

MLA pre-treatments: On reinstatement day (day 19) animals were randomly allocated to one of 4 treatment groups. Group A: received saline (10ml/kg, s.c.) followed by a priming dose of morphine (5mg/kg, i.p.); group B: received MLA (4mg/kg, s.c.) followed by a priming dose of morphine (5mg/kg, i.p); group C: received MLA (4mg/kg, s.c.) followed by a saline priming dose; group D: received saline (10ml/kg, s.c.) followed by a priming dose of saline (10ml/kg, i.p.).

Mecamylamine pre-treatments: On reinstatement day (day 19) animals were randomly allocated to one of 4 treatment groups. Group A: received saline (10ml/kg, s.c.) followed by priming dose of morphine (5mg/kg, i.p.); group B: received mecamylamine (1mg/kg, s.c.) followed by a priming dose of morphine (5mg/kg, i.p). group C: received mecamylamine (1mg/kg, s.c.) followed by a saline priming dose; group D: received saline (10ml/kg, s.c.) followed by a priming dose of saline (10ml/kg, i.p.).

Rat Procedures

For experiments to investigate the effect of MLA on the acquisition and reinstatement of morphine-CPP, rats were treated in the same way with exceptions to the doses (all administered s.c.). Morphine conditioning doses were delivered at 5mg/kg (s.c), and reinstatement priming doses at 2.5mg/kg (s.c). MLA was delivered at 4mg/kg (s.c).

At the end of all behaviour CPP experiments animals were killed by cervical dislocation and in some cases brain were taken for biochemistry and stored at 20°C, or frozen in isopentane for autoradiography (see section 2.4).

Mecamylamine dose validation

To test the effect of mecamylamine on locomotor activity to nicotine animals received daily nicotine pairings. During the pairing phase (days 1-6) mice received daily s.c injections of the following, either: saline + saline, saline + nicotine (0.175mg/kg), mecamylamine (1mg/kg) + saline, or mecamylamine (1mg/kg) + nicotine (0.175mg/kg). The first injection was given 20 minutes prior to the second in the holding room. Immediately after the second injection animals were placed into the locomotor apparatus (a square box measuring 30x30cm sat on a textured lino) and the activity recorded for 30mins. 5 days later all animals were injected with the challenge dose of nicotine (0.175mg/kg), and their activity was recorded for 30 minutes. At the end of the test animals were killed by cervical dislocation.

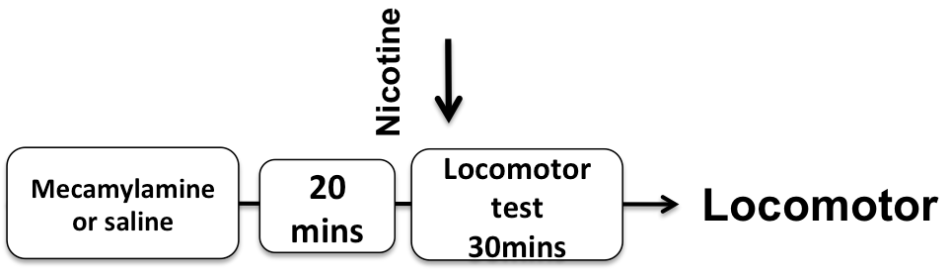


Figure 2-2 The protocol for locomotor dose validation.
The whole protocol as shown was repeated for 6 days (the pairing phase). During the pairing phase (days 1-6) mice received daily s.c injections of the following, either: saline + saline, saline + nicotine (0.175mg/kg), mecamylamine (1mg/kg) + saline, or mecamylamine (1mg/kg) + nicotine (0.175mg/kg). The first injection was given 20minutes prior to the second in the holding room. Immediately after the second injection animals were placed into the locomotor apparatus and the activity recorded for 30mins. 5 days later all animals were injected with the challenge dose of nicotine (0.175mg/kg), and their activity was recorded for 30 minutes.

2.3 Intra cerebral cannula implantation and drug delivery

Experimental design

Before surgery male Wistar rats (350-400g) had 2 habituation baseline tests (1x15 minutes/day), four conditioning sessions (1x40 minute/day) followed by post-test (1x15minutes), 4 extinction sessions (1x30 minute/day), and a further post-test (2) (1x15minute) (figure 2.4). Morphine (5mg/kg, s.c) or saline (10ml/kg, s.c) were given on alternate conditioning days. No exclusions were made. Animals were then pseudo-randomised for treatments balanced across acquisition and extinction of morphine-CPP, into one of 3 groups of infusion sites: the medial prefrontal cortex (mPFC), dorsal (dHPC) or ventral hippocampus (vHPC) for surgical implantation of cannula.

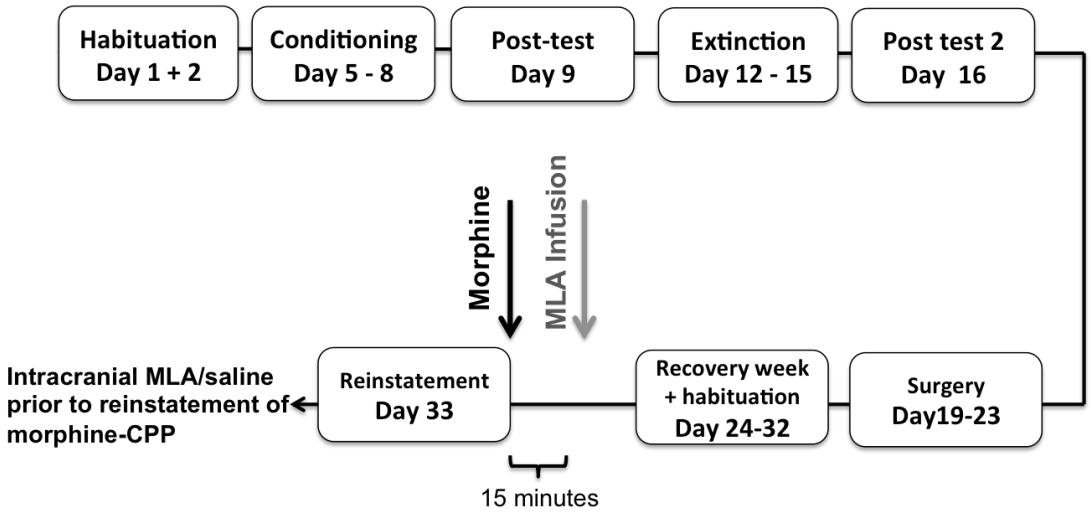


Figure 2-3 The protocol for the intracranial cannulation experiment.
Before surgery male Wistar rats (350-400g) had 2 habituation baseline tests (1x15 minutes/day), four conditioning sessions (1x40 minute/day) followed by post-test (1x15minutes), 4 extinction sessions (1x30 minute/day), and post-test 2 (1x15minute)

Validation of procedure

Initial experiments were conducted to ensure that the surgery had no effect on the reinstatement of morphine-CPP, after which 3 final experiments were grouped (as described in table 2.1). For the validation 12 Wistar rats underwent conditioning and extinction training. 6 animals underwent cannulation surgery (as described in section below) (2 mPFC, 2 dHPC, 2vHPC placements) and were allowed 1 week for recovery, whilst 6 animals remained in their home cages. Cannulated animals then underwent infusion procedures with saline, whilst control animals were given saline (10ml/kg,s.c) 20 minutes prior to their morphine priming dose (2.5mg/kg, s.c) and reinstatement trial.

MLA intracerebral procedure (experiments 1-3)

52 Wistar rats underwent conditioning and extinction training (shown in grey in table 2.1). Then all animals underwent cannulation surgery (as described in below) (16 mPFC, 19 dHPC, 18 vHPC placements) and were allowed 1 week for recovery, 3 of these animals did not recover adequately and were killed by rising levels of CO^2. Cannulated animals then underwent infusion procedures (outline in detail in below) with MLA (6.74μg/hemisphere) (with saline controls receiving identical volumes), 20 minutes prior to their morphine priming dose (2.5mg/kg, s.c and reinstatement trial.

Table 2-1 The treatments and experimental outcomes of the intracranial infusion experiments

Experiment	n	Treatments	Experimental losses
Validation	6 non surgery controls 6 surgery	N/A 2 mPFC, 2 dHPC, 2 vHPC	 No losses
1	16	6 mPFC, 5 dHPC, 5 vHPC	No losses
2	16	5 mPFC, 5 dHPC, 4 vHPC	1 loss
3	20	5 mPFC, 7 dHPC, 7 vHPC	2 losses

Surgical procedures

Following acquisition and extinction of morphine-CPP, but prior to the reinstatement test rats were anaesthetised with isofluorane (induction 4%, maintenance, 2-3%, Baxter, UK) and the surgery site was shaved and cleaned with ethanol wipes. The rat was then placed into a stereotaxic frame on a heat mat to maintain a consistent body temperature throughout the procedure. After the skull was exposed two dorsal-ventral (D-P) measurements were taken one at lambda and another at bregma, the incisor bars were set accordingly to achieve a flat skull position. Burr holes were drilled and the guide cannulae were implanted at coordinates relative to bregma; to target the mPFC: anterior-posterior +3.20, medial-lateral ±0.75, dorsal-ventral -2.8; the dorsal hippocampus: anterior-posterior +3.20, medial-lateral ±2.5, dorsal-ventral -1.8; ventral hippocampus: anterior-posterior -5.3, medial-lateral ±5.2, dorsal-ventral -4.5 (figure 2.5). The implanted cannulae were anchored to the skull with three stainless steel screws (Plastics One, Semat, UK) and dental cement. Dummy cannula were placed in the guide cannulae and secured with a dust cap to prevent post-surgical infection. The wound was sutured, antiseptic iodine spray (Savlon) was applied. The rats were rehydrated with 0.9% saline solution (s.c10ml/kg), given 0.2ml antibiotic (s.c, Clamooxyl LA. Pfizer 150mg/ml) and postoperative pain relief was given (Caprieve 5mg/kg, s.c). All animals were given 1 week to recover following surgery. Daily checks were made and the animals were habituated to the infusion processes.

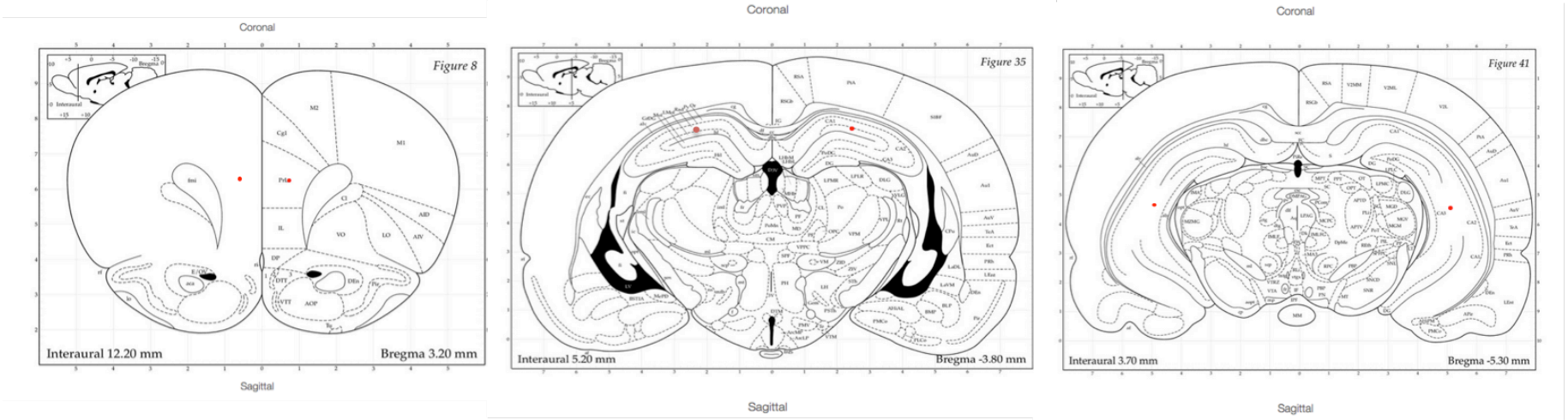


Figure 2-4 The stereotaxic coordinates (shown in red) for the implantation of bilateral indwelling cannulae.
The guide cannulae were implanted at coordinates relative to bregma (from left to right): the mPFC: anterior-posterior +3.20, medial-lateral ±0.75, dorsal-ventral -2.8; the dorsal hippocampus: anterior-posterior +3.20, medial-lateral ±2.5, dorsal-ventral -1.8; ventral hippocampus: anterior-posterior -5.3, medial-lateral ±5.2, dorsal-ventral -4.5. Coordinated determined using a rat brain atlas (Paxinos and Watson, 2007).

Infusion

Compounds administered were dissolved in a 0.9% saline solution and made up on the morning of the infusion. After removal of the cap and dummy cannula, a 33-gauge infusion cannula, connected to a 25µl Hamilton syringe (Hamilton, UK) by polyethylene tubing (0.50mmx0.50mm, SLS, UK), was inserted into the guide cannula. The infusion pump (Harvard apparatus) was switched on and used to slowly infuse 2.4µl/hemisphere over a 4-minute period. Methyllycaconitine (MLA, Tocris UK) was administered at a dose of 6.74µg/hemisphere (with saline controls receiving identical volumes). The infusion pump was switched off and the infusion cannulae were left in place for a further 4 minutes before removal and replacing the dummy and dust cap. Infusions were conducted in pairs of animals 15 minutes before the reinstatement trial.

Dissections and cannula placement verification

Animals were sacrificed by rising concentrations of CO_2 immediately after behavioural testing and then all cannulae placements were verified by an infusion of 0.5ul of brilliant blue dye using the infusion pump. The brains were removed and frozen in isopentane on dry ice, and temporarily stored in liquid nitrogen before being moved to -80°C freezer. The brains were mounted onto a cold stage with Optimal cutting temperature compound (OCT) and placed into a cryostat cooled to -21°C. Using a rat atlas as a guide (Paxinos and Watson, 2007) frozen coronal sections were made until the dye marks were reached. The section containing the dye mark was photographed and superimposed onto the corresponding bregma of the brain atlas. The infusion site was then added to the schematic showing all placements for the corresponding treatment.

2.4 Autoradiography

Sectioning

All mice underwent behavioural protocols as outlined in section 2.2. After reinstatement animals were sacrificed by cervical dislocation and the brains were removed and frozen in isopentane on dry ice, temporarily stored in liquid nitrogen before being moved to a -80°C freezer. Prior to sectioning the brains were mounted on a cold stage with OCT and placed in the cryostat (Leica, UK) cooled to -21°C. Using a mouse atlas as a guide (Franklin & Paxinos, 1997) adjacent frozen coronal sections, 20uM thick from 4 regions: The level of the prefrontal cortex (bregma 1.94mm), the striatum (bregma 1.42mm), the dorsal hippocampus (bregma -1.22mm) and the mid-brain/ventral hippocampus (bregma -3.08mm). Consecutive sections were cut and freeze-thaw mounted onto separate gelatin-subbed glass slides (Thermo Scientific) for total and non-specific labelling. Slides were dried in slide holders sealed in boxes containing anhydrous calcium sulphate (Drierite) and placed in the fridge for 2 hours. Boxes were stored at -80°C for at least 3 days.

Glutamate receptor autoradiography

NMDA receptor autoradiography

Tritiated MK801 binding (specific activity: 22.5 Ci/mmol, Sigma, Poole, Dorset) was used to determine the levels of NMDA receptors in a protocol previously described by Reynolds (2001). The slides stored at -80°C, were left at room temperature for 30mins to thaw. The slides were then incubated for 20mins at room temperature in buffer (50mM tris-HCL, pH 7.4 containing 50μM glutamate, 50μM glycine and 50μM spermidine) to remove endogenous ligands. Subsequently slides were incubated for 1 hour at 4 °C in buffer with 70nM [^{3}H]-(+)-MK801 to determine total binding. Non-specific binding was determined in the presence of 1mM (+)-MK801. The slides were rinsed twice for 30 seconds in ice-cold tris buffer (50mM tris-HCL, pH7.4) and briefly submerged in ice-cold water. The slides were then rapidly dried with a stream of cool air and placed in a sealed container with anhydrous calcium sulphate for 1 week before being apposed to film for 3 weeks.

AMPA receptor autoradiography

Tritiated AMPA autoradiography was conducted as previously described by (Duncan *et al.*, 2002). Sections were pre-incubated in 50mM Tris buffer (tris-HCL, pH7.4) containing 50mM sodium thiocyanate for 20 minutes at room temperature. Total binding was determined by incubating sections in the same buffer containing 10nM [^{3}H]-AMPA (specific activity: 58.1 Ci/mmol, Perkin Elmer Life Sciences, USA) for 45 minutes at room temperature. Adjacent sections were incubated in the additional presence of 0.1mM CNQX (Sigma, UK) to determine non-specific binding. Sections were washed for a total of 60 seconds in three changes of ice-cold buffer before being briefly rinsed in distilled water and dried in a stream of cool air and placed in a sealed container with anhydrous calcium sulphate for 1 week before being apposed to film for 4 weeks.

Autoradiographic film apposition and film development

Slides were apposed to Kodak MR-1 films (Sigma-Aldrich, UK) in Hypercassettes with autoradiographic [^{3}H] microscales of known radioactive concentration (GE Healthcare Life Sciences, Amersham, U.K.) for 3 weeks. Sections for all treatment groups were processed in parallel and apposed to the same film at the same time. Film development was carried out in the dark under red-filtered light, in a 50% Kodak D19 developer solution (Sigma-Aldrich, Poole, UK) for 3 minutes. To stop the development reaction the films were then washed in distilled water containing glacial acetic acid for 30 seconds followed by 5 minutes fixation step in a Kodak rapid fix solution (Sigma-Aldrich, Poole, UK). Finally, films were rinsed in cold running water for 20 minutes and left to dry in a fume hood.

Image analysis for quantitative autoradiography

Quantitative autoradiographic analysis of all structures were carried out by reference to the mouse brain atlas of Franklin and Paxinos (1997) and binding was analysed as previously described (Bailey *et al.*, 2010), using MCID image analyser (Image Research, Ontario, Canada). The regions that were quantified are outlined in figure 2.6. Briefly, optical density values, which were quantified from autoradiographic [^{3}H] microscales of known radioactive concentration (GE Healthcare, UK), were entered with their corresponding radioactivity values into a calibration table, and the relationship between radioactivity and optical density was subsequently determined using the MCID software. Specific binding was determined by subtracting the NSB from the total binding in the images of the brain sections. For [^{3}H]-AMPA NSB was homogeneous therefore representative NSB area was subtracted from all total values, but for [^{3}H]-(+)-MK801 NSB was taken from corresponding area for each brain region analysed.

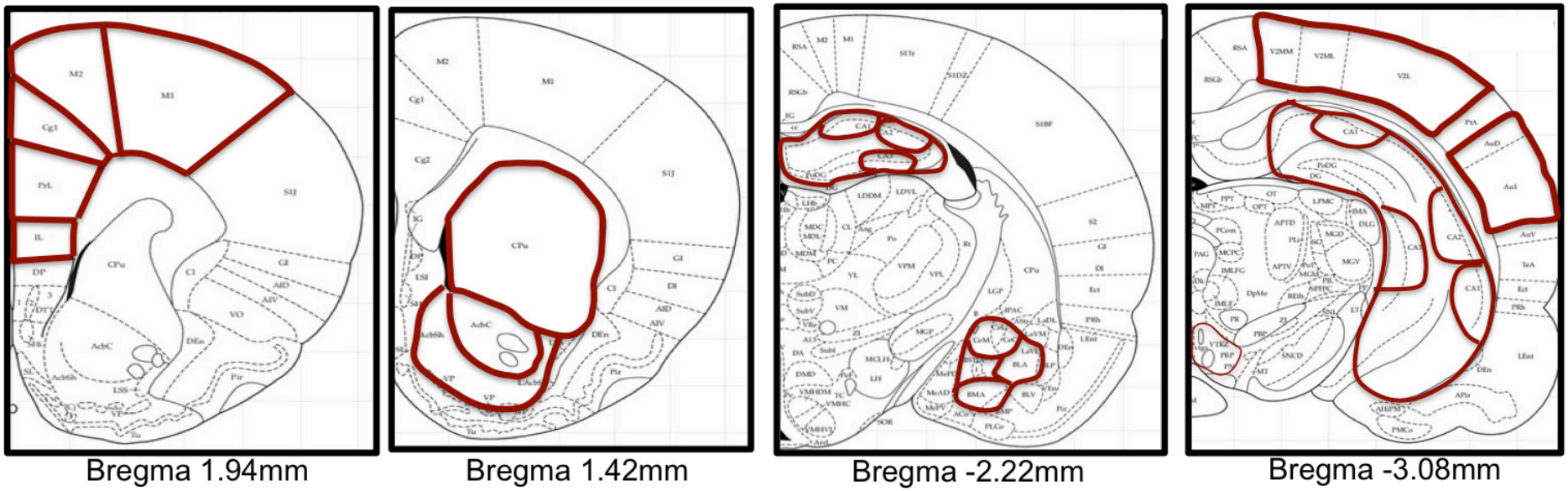


Figure 2-5 Image analysis for quantitative autoradiography showing areas analysed.
Schematics of each bregma and the areas that were quantified using MCID software. Bregma 1.94mm: Prelimbic (PrL) and infralimbic (IL) areas of the mPFC; the cingulate cortex (Cg1), the motor cortices (M1 and M2). Bregma 1.42mm: the caudate putamen (CPu), accumbens core (NAcc), accumbens shell (NAcs). Bregma -2.22mm: Dorsal hippocampus (dHPC), including subregions CA1-3 (dCA1-3), amygdala: including basomedial amygdala (BMA), central amygdala (CeA) and the basolateral amygdala (BLA). Bregma -3.08mm: Ventral hippocampus (vHPC), including subregions CA1-3 (vCA1-3), visual cortex (ViCx 1-2), and Auditory cortex (AuCx 1-2).

CHAPTER 3 THE ROLE OF NACHRS IN MORPHINE CONDITIONED PLACE PREFERENCE

3.1 Behavioural effects of nAChRs in morphine reward learning

One of the strongest predictors of opioid abuse is early life tobacco and alcohol use (Woodcock *et al.*, 2015) and cross sensitization between nicotine and morphine has been reported (Vihavainen *et al.*, 2008) in animal studies. Morphine administration can reverse nicotine withdrawal in rats (Ise *et al.*, 2000) and morphine's enhancing effects on locomotion are potentiated by nicotine treatment (Biala & Weglinska, 2004; Vihavainen *et al.*, 2006). Furthermore nicotine enhances morphine's antinociception effect in mouse-tail flick experiments (Suh *et al.*, 1996) and nicotine improves morphine-induced impairment of memory (Ahmadi *et al.*, 2007) suggesting a close interaction between the two drug systems.

The phenomena of cross-reinstatement, the ability of drugs other than those previously received to reinstate drug seeking behaviour, has been described extensively (Biala & Budzynska, 2006). Recently it has been demonstrated that nicotine induced-CPP can be reinstated by morphine priming in mice (Biala *et al.*, 2010). Furthermore bupropion, an antidepressant that affects several different targets but appears to have non-competitive antagonist activity at nAChRs, can block nicotine induce reinstatement of nicotine CPP (Budzyńska & Biała, 2011) reinforcing its use as a smoking cessation agent (Wilkes, 2008). However, to date nicotine has not been shown to reinstate morphine CPP (Feng *et al*, 2011), but acute nicotine priming does modulate responses to morphine CPP in mice (Zarrindast *et al.*, 2003; Vihavainen *et al.*, 2008a). Vihavainen *et al* (2008) report a similar augmentation of the reinforcing properties of morphine in mice and show that this cross sensitisation is not mediated by μ opioid receptors as they found no change in [^{3}H]DAMGO binding following chronic nicotine treatment.

Both morphine and nicotine increase DA release in the terminal areas of the mesolimbic DA neurons (Di Chiara & Imperato, 1988; Shippenberg *et al.*, 1993). μ-opioid receptors on GABAergic neurons in the VTA and SN are thought to mediate morphine induced DA release (Johnson & North, 1992) through inhibition, resulting in an increase in dopaminergic firing. Chronic nicotine treatment has been shown to modify GABAergic control of dopamine neurons (Vihavainen *et al.*, 2008b). Morphine induced elevation of DOPAC, a metabolite of DA, increased in the CPu of

mice chronically treated with nicotine (Vihavainen *et al.*, 2006)**,** which suggests an increase in DA turnover.

As discussed in Chapter 1, other than DA levels, there is experimental evidence emerging that suggest that rising levels of endogenous acetylcholine may also play an important role in reward reinforcement. Levels of intra-accumbal ACh have been shown to increase after morphine (Fiserová *et al.*, 1999; Crespo *et al.*, 2006) cocaine (You *et al.*, 2008), ethanol (Imperato *et al.*, 1998), after lever pressing for food rewards (Orsetti *et al.*, 1996), exposure to reward associated cues (Pych *et al.*, 2005; Goldberg & Reynolds, 2011), but not sucrose self-administration (Crespo *et al.*, 2006). Inactivation of the primary input of ACh in to the VTA, the PPT has been shown to abolish pair stimulus reward learning, conditioned reinforcement (Inglis *et al.*, 2000) and also impairs the ability of the VTA DA neurons to burst fire in the presence of reward-predictive cues.

Whereas nicotine acts through nAChRs, endogenous acetylcholine also acts through muscarinic receptors. Therefore the role of acetylcholine signalling in reward has prompted research into determining a site of action for this effect. Acetylcholine acting through muscarinic acetylcholine receptors may be necessary for drug conditioning (Rezayof *et al.*, 2006) but only muscarinic receptors seem to be important in reward responding for food (Crespo *et al*, 2006).

Whilst the muscarinic receptors appear to have an effect on both natural rewards, such as food, and drug rewards, the nicotinic effect seems to be more specific to drugs of abuse (Crespo *et al*, 2006). Non-specific antagonists of the nAChR, such as mecamylamine have been shown to attenuate locomotor sensitisation to morphine (Biala & Staniak, 2010) and intra hippocampal administration of mecamylamine inhibits morphine induced CPP (Zarrindast *et al.*, 2003). The effect of specific nAChR subtypes is less thoroughly investigated. Feng *et al* (2011) have shown that antagonising either $\alpha4\beta2$ or $\alpha7$ nAChR subtypes, with DHβE and MLA respectively, blocks reinstatement to morphine-induced CPP by drug priming in Balb/c mice.

However there are reports that have demonstrated that a hypothesised increase in acetylcholine transmission, induced by systemic administration of acetylcholinesterase inhibitors, also reduces the development of morphine (Gawel *et al.*, 2014), cocaine and heroin-induced, (Zhou *et al.*, 2007; Liu *et al.*, 2011) reinstatement to CPP. However, Zhou et al (2007) showed that the effect was reversed by a pre-treatment of scopolamine, a muscarinic antagonist, but not mecamylamine, suggesting the increase in acetylcholine signalling reduced heroin responding through a muscarinic mechanism. Furthermore the increase in the rate of self-administration they saw with scopolamine may have been to overcome the reduced rewarding properties of each heroin injection, rather than an increase in the rewarding value of heroin. Critically an increase in acetylcholine was not actually measured in these studies; therefore it is unclear where the drug is acting. Finally lobeline, an atypical agonist at nAChRs was shown to attenuate self-administration of heroin (Hart *et al.*, 2010) and varenicline, a partial agonist, was shown to attenuate the locomotor sensitisation to morphine (Biala & Weglinska, 2004). However, it is likely that this effect is through lobeline's ability to antagonise the μ opioid receptor (Miller *et al.*, 2007), rather than its agonist activity at α4β2 nAChRs.

Overall preclinical evidence suggests that nAChRs are important modulators of drug reinforcement, and specific receptors seem to have different effects on responses to different psycho-stimulants. For example α4β2* and α3β4* may be important in alcohol and nicotine seeking, whilst only muscarinic receptors play a part in the reinforcement to food and sucrose (Yeomans & Baptista, 1997; Lê *et al.*, 2000; Levin *et al.*, 2000; Sharf & Ranaldi, 2006; Champtiaux *et al.*, 2006; Chatterjee *et al.*, 2011). More research is needed on the role of α7 nAChRs receptors in morphine reward, as an effect has been demonstrated in Balb/c mice on reinstatement to morphine-CPP, but not other stages of the CPP paradigm. Particular interest has fallen on the α7 receptor due to its high permeability to Ca^{2+} (Fucile *et al.*, 2005), which enables the receptor to modulate neurotransmitter release and influence gene expression of early immediate genes related to memory and learning (Carlezon & Nestler, 2002). Drug associated learning is thought to be increasingly important in relapse to drugs of abuse and there is research relating the α7 receptor in cue retrieval

as well as memory and learning (Felix & Levin, 1997; Nott & Levin, 2006; Gu *et al.*, 2012) make it an excellent target for modulating drug responses in CPP.

3.2 Aims of Chapter

The aim of this chapter was to determine the role of nAChRs in the different stages of morphine-CPP, a model of drug association learning and relapse. The focus of this study was the α7 nAChR due to involvement in downstream events relating to learning and memory (Nestler, 2002)**.** The subtype-selective antagonist methyllycaconitine (MLA), was used as it has well documented antagonistic action at the α-bungarotoxin binding site (Ward *et al.*, 1990)**,** which are known to reside primarily on α7 receptors (Marks *et al.*, 1999)**.** We selected a dose of 4mg/kg s.c previously used in the literature (Feng *et al.*, 2011; Andriambeloson *et al.*, 2014)**.**

3.3 Results

Effect of systemic MLA on morphine-induced acquisition and reinstatement

In this series of experiments, the CPP protocol developed in Appendix A was used. Acquisition, reconsolidation, expression and reinstatement of morphine-CPP were examined using separate cohorts of animals.

The effect of MLA on the acquisition of morphine-CPP

To investigate whether MLA can inhibit acquisition of morphine-induced CPP animals were randomly allocated to one of four treatment groups, after habituation to the CPP apparatus. Either saline (10ml/kg, s.c.) immediately prior to morphine (10mg/kg, i.p.) or saline; MLA (4mg/kg, s.c.) immediately prior to morphine (10mg/kg, i.p) or saline (10ml/kg, i.p) before each conditioning trial (Figure 3.1). A repeated measures one-way ANOVA showed no significant effect of treatment ($F_{(1,22)}$=0.15, p=0.699) but a significant effect of test ($F_{(1,30)}$=24.09, p=<0.001) showing an effect of morphine conditioning. Post-hoc pairwise comparisons revealed significant morphine-CPP in saline + morphine group (preference score during habituation was 10.8±39.0 vs. 164.1±34.5 post conditioning, n=16, p=0.012), and the MLA+morphine group (preference score during habituation was -3.2±36.7 vs. 179.8±45.8 post conditioning, n=16, p=0.003). There was no significant difference between the MLA pretreated and the saline pretreated groups (n=16, p=0.69). Figure 3.1B shows that MLA alone induced no CPP (MLA + saline: habituation 3.7±28.6, post conditioning 33.1±40.7 vs saline + saline: habituation 18.3±52.9, post conditioning 16.2±41.0, no effect of treatment *p*=0.75 or test p=0.45, n=7/treatment group). Showing MLA pretreatment has no effect on the acquisition of morphine CPP.

Figure 3.1C shows that morphine treatment had a significant effect on locomotor activity as measured by distance moved (ANOVA with type two model fit revealed $F_{(3,28)}$=8.65, *p*=<0.001*)*. Morphine significantly increased distanced moved (Pairwise comparisons with Benjamini-Hochberg test, saline and saline 2327±206cm vs saline and morphine 7561±1169cm, n=8/treatment, p=0.001). Morphine-induced locomotor activity was still evident following MLA pre-treatment

(5878.0±1389.7cm, n=8/treatment, 4mg/kg, i.p, p=0.025), this indicates that MLA has no significant effect on morphine-induced locomotion. MLA administered alone had no effect on locomotor baseline activity (saline and saline: 2327.0±206.9cm vs MLA and saline 2094.8±115.6cm, p=0.859, n=7/treatment.).

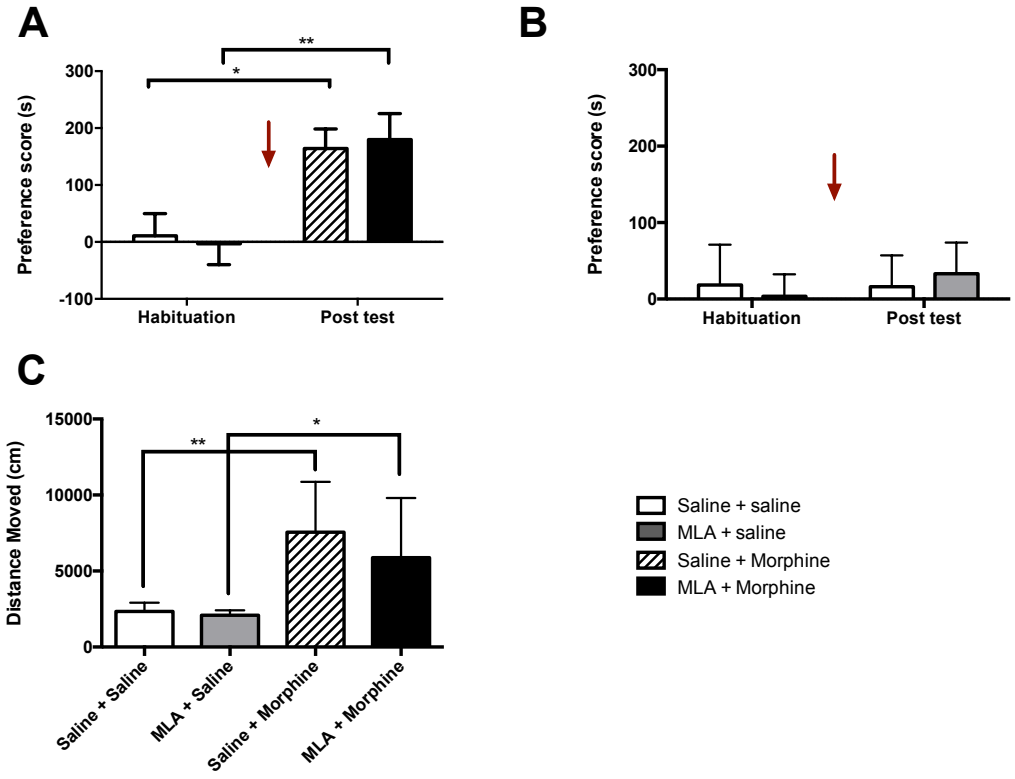


Figure 3-1 The effect of MLA on the acquisition of morphine-CPP.
A) Animals were treated with 4 mg/kg, s.c, MLA (arrow) 20mins prior to the morphine conditioning dose (10mg/kg, i.p; arrow). Time spent in DP side was measured over the 15min post test period (data shown as mean± SEM). A repeated measures ANOVA reveal no significant difference between the time spent in the drug paired side after conditioning in the MLA pretreated and the saline pretreated groups (n=16, p=0.69), both groups showed significant acquisition of morphine-CPP (Saline: *p=0.012, MLA: **p=0.003 n=16/treatment. **B)** Animals were treated with either saline or MLA (arrow, hatched bars indicated post MLA treatment) before a saline conditioning dose (10ml/kg, i.p). A repeated measures ANOVA showed no difference in the time spent in the drug paired side. **C)** The effect of MLA pretreatment on locomotor activity, measure via distance moved. Morphine treatment significantly increased distance traveled in both saline (**p=0.001) and MLA (*p=0.025) pretreated animals. There was no difference of MLA pretreatment on saline locomotor activity (p=0.859).

The effect of MLA on the reconsolidation of morphine CPP.

To test whether MLA has an effect on the reconsolidation of a memory previously formed during CPP animals were placed in the DP side after a further morphine (10mg/kg, i.p) dose, then MLA (4mg/kg, s.c) was given immediately after this reconsolidation trial in the experimental room. Figure 3.2 shows the effect of MLA on the reconsolidation of morphine-CPP. An ANOVA with repeated measures revealed no significance in the effect of treatment ($F_{(1,17)} = 0.1$, p=0.961) but a significant effect of test ($F_{(2,44)} = 14.25$, p=<0.001, n=12/treatment group. *Post hoc* analysis with Benjamini-Hochberg test revealed no significant difference between treatment groups after the reactivation trial (p=0.981) or one week later, indicating that MLA had no effect on reconsolidation of morphine-induced CPP.

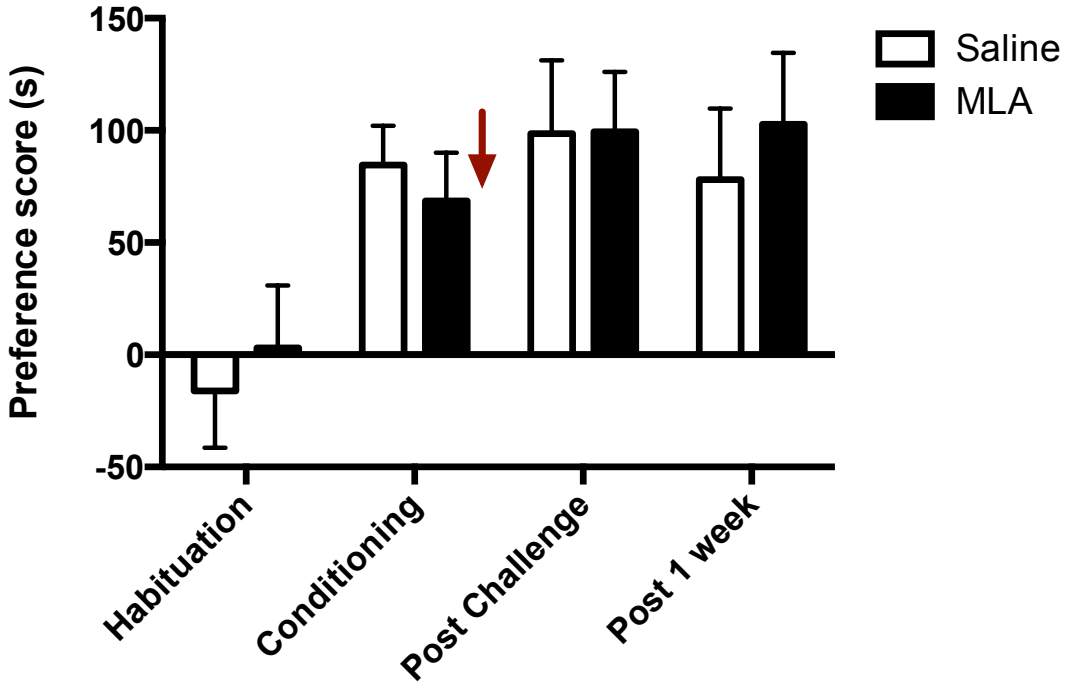


Figure 3-2 The Effect of MLA (4mg/kg, s.c) on the reconsolidation of morphine-CPP.
Animals underwent morphine conditioning, the following day animals were treated with MLA (4mg/kg, s.c) immediately after a 30min session in the DP side (arrow). The animals were then tested for the preference on the following day (post challenge) and a week later (Post 1 week). All data shown as mean± SEM. There was no difference in the time spent in the drug paired side 1 day after the challenge or 1 week later, compared with the saline pre-treated animals. There was a significant effect of test showing CPP was acquired in both groups (p=<0.001, n=12/treatment group).

The effect of MLA on the expression of morphine CPP.

To test whether MLA has any effect on the expression, the ability to express the previously acquired association, of morphine CPP animals were given MLA (4mg/kg, s.c) 20 minutes prior to each post-test session for 4 days and 1 week after the first post-test session (figure 3.3). A repeated measures ANOVA revealed a significant effect of test ($F_{(6,78)}$=4.37, p=0.001, n= 12/treatment) but not of treatment ($F_{(1,13)}$=0.78, p=0.394, n=12/treatment). *Post hoc* pairwise comparisons with Benjamini-Hochberg test revealed significant expression of morphine CPP on post-test day (saline: p=0.004, MLA: p=0.024, n=12/treatment). These data show that MLA pre-treatment has no effect on the expression of morphine-CPP

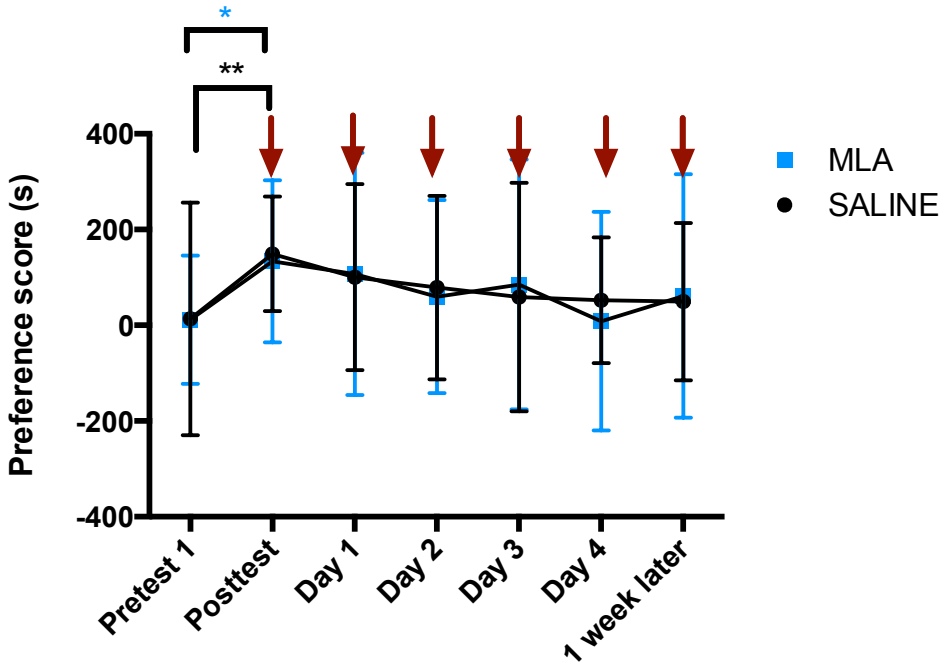


Figure 3-3 The effect of MLA on the expression of morphine-CPP.
To test whether MLA has an effect on the expression of morphine CPP animals were given MLA (4mg/kg, s.c (arrow)) 20 minutes prior to each post test for 4 days and 1 week after the first post test (data shown as mean± SEM). A repeated measures ANOVA revealed a significant effect of test ($F_{6,78}$=4.37, p=0.001, n= 12/treatment) but not of treatment ($F_{1,13}$ =0.78, p=0.394, n=12/treatment), showing morphine conditioning but no effect of MLA pre-treatment.

The effect MLA on the reinstatement of morphine CPP.

To test whether MLA has an effect on the reinstatement of morphine-CPP by morphine drug priming, morphine CPP was first established, then extinguished with repeated saline injections before a drug-primed reinstatement test was conducted. MLA (4mg/kg, s.c) was administered 20 min prior to the morphine or saline priming reinstatement dose. Figure 3.4 shows the effect of MLA on the reinstatement of morphine-CPP. Three separate experiments were conducted and pooled to compensate for the larger variability seen in reinstatement data (see appendix A). A repeated measures one-way ANOVA revealed no significance in the effect of treatment ($F_{(1,52)} = 1.15$, p=0.288) but a significant effect of test ($F_{(3,156)} = 13.48$, p=<0.001, n=20/treatment group). *Post-hoc* analysis with Benjamini-Hochberg test for multiple comparisons revealed only animals pre-treated with saline significantly reinstated (saline: 42.2±.14.0s in DP paired side at extinction vs 143.1±33.2s at reinstatement p=0.003; MLA: 37.3±13.8s in DP paired side at extinction vs 67.3.4±41.7s at reinstatement, p=0.135, n=20/treatment group). The time spent in drug paired side was significantly different between the two treatments (p=0.0016). These data show that MLA significantly inhibits reinstatement to morphine-CPP.

There was a significant effect of treatment on locomotion. A one-way ANOVA revealed a significant effect of treatment (Figure 3.4B: F= 5.2, p=0.026, n=20/treatment group), demonstrating that MLA significantly reduced locomotion.

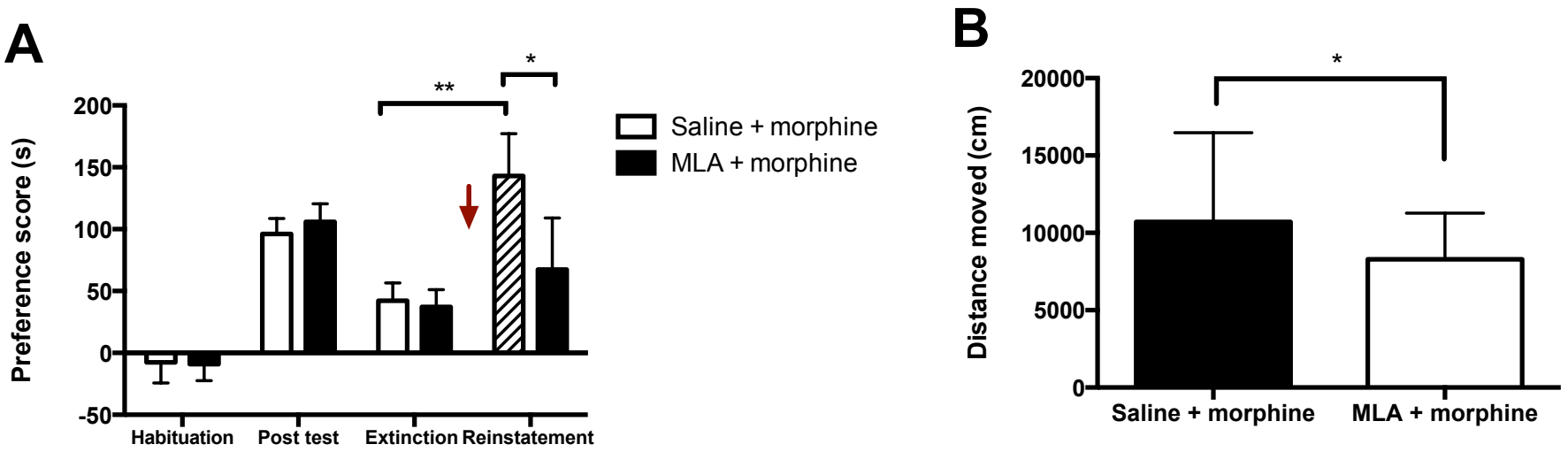


Figure 3-4 The effect of MLA pre-treatment on reinstatement of morphine-CPP.
A) MLA (4mg/kg, s.c) was administered 20 minutes prior to the morphine priming dose (arrow/hatched bars indicated post MLA treatment). All data shown as mean± SEM. Post-hoc analysis with Benjamini-Hochberg test for multiple comparisons revealed only animals pre-treated with saline significantly reinstated (saline: 42.2±.14.0s in DP paired side at extinction vs 143.1±33.2s at reinstatement **p=0.003; MLA: 37.3±13.8s in DP paired side at extinction vs 67.3.4±41.7s at reinstatement, p=0.135, n=20/treatment group) The time spent in drug paired side was significantly different between the two treatments (*p=0.0016). B) The effect of MLA pre-treatment on locomotion. There was a significant effect of treatment on locomotion. A one-way ANOVA revealed a significant effect of treatment (F= 5.2, p=0.026, n=20/treatment group).

MLA (4mg/kg, s.c) significantly reduces reinstatement to morphine CPP but has no effect on the acquisition, expression, or reconsolidation of CPP.

To summarise, the effect of MLA on CPP seems to be limited to partially inhibiting reinstatement, and has no effect on the acquisition, expression or reconsolidation of morphine-CPP. To test whether this effect was specific to this nAChR subtype the next section aimed to establish whether mecamylamine, a non-specific antagonist, had an effect on acquisition and reinstatement of morphine-CPP.

The effect of mecamylamine on morphine-CPP

Dose validation

A preliminary experiment was run to select a dose for mecamylamine (figure 3.5) to ensure sufficient brain concentrations were acquired to test for effect on CPP. The effect of mecamylamine at 1 mg/kg, a dose used previously in mice (Zachariou *et al.*, 2001; Neugebauer *et al.*, 2013) was investigated on the locomotion effects of nicotine, using the paradigm outline in the methods section (2.2). As this behavioural phenomenon is centrally mediated (Wise & Bozarth, 1987) any interference from mecamylamine would implicate an action at central nAChRs. An ANOVA with repeated measures revealed a significant effect of test ($F_{6,96}$=9.23, p= <0.001, n=5/treatment) and treatment ($F_{3,16}$=5.29, p=0.01, n=5/treatment). *Post hoc* pairwise comparisons revealed nicotine significantly decreased locomotor activity at day 2-5 (p=0.018, 0.014, 0.006, 0.017, saline nicotine vs saline saline, n=5/treatment). Mecamylamine blocked nicotine's effect on locomotion on Day 1-6 (p=0.003, 0.001, 0.002, <0.001, 0.001, 0.007, Mecamylamine+nicotine vs saline + nicotine, n=5/treatment). This reveals that 1mg/kg mecamylamine is an appropriate dose as it significantly blocks the locomotor inhibition shown by nicotine treatment.

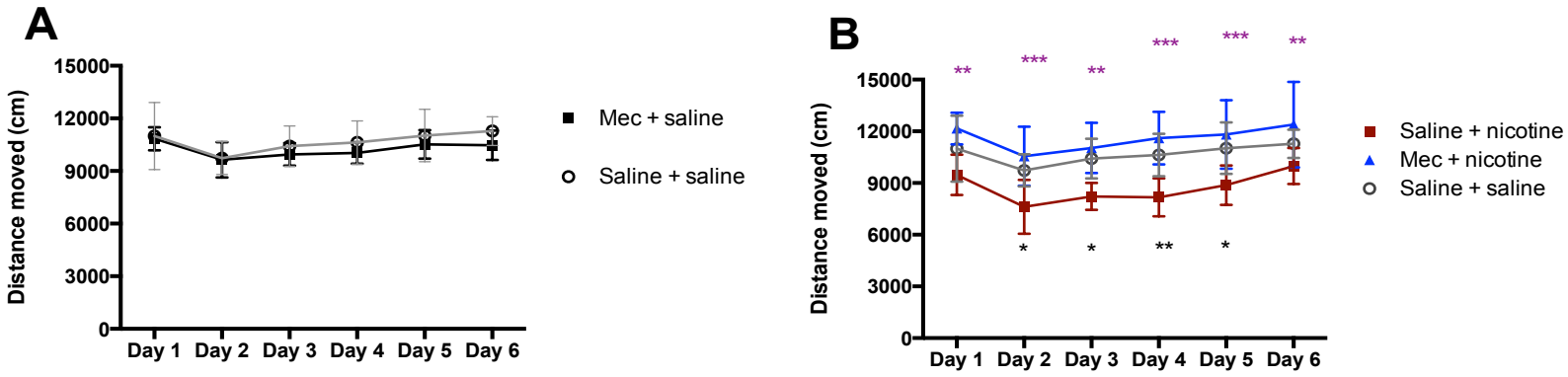


Figure 3-5 The effect of mecamylamine on nicotine suppression of locomotion.
Pre-treatment of either mecamylamine (1mg/kg, s.c) or saline (10ml/kg, s.c) was given before a nicotine (0.175mg/kg, s.c) or saline dose (10ml/kg) and exposure to a locomotor arena for 40 minutes (data shown as mean ± SEM). **A)** Mecamylamine pre-treatment had no effect on distance moved in the absence of nicotine **B)** Post hoc pairwise comparisons revealed nicotine significantly decreased locomotor activity at day 2-5 (p=0.018, 0.014, 0.006, 0.017, saline nicotine vs Saline saline, n=5/treatment). Mecamylamine significantly reduced nicotine's effect on locomotion on Day 1-6 (p=0.003, 0.001, 0.002, <0.001, 0.001, 0.007, Mecamylamine+nicotine vs Saline + nicotine, n=5/treatment).

The effect of mecamylamine on acquisition of morphine-CPP

The effective dose of 1mg/kg mecamylamine s.c was used to determine any effect of the drug on the acquisition of morphine-CPP. Mice were randomised into one of four treatment groups: mecamylamine+morphine, mecamylamine+saline, saline+morphine or saline+saline. Mice were given either saline (10ml/kg, s.c) or mecamylamine (1mg/kg, s.c) 20 minutes prior to the morphine or saline conditioning dose daily for 4 days. Results of the preference test are shown in Figure 3.6. A one-way ANOVA with repeated measures revealed a significant effect of test ($F_{1,10}$=6.67, p=0.0275, n=8/treatment) but not treatment ($F_{1,10}$=3.31, p=0.099, n=8/treatment). Pairwise comparisons with Benjamini-Hochberg's correction showed significant acquisition of CPP in both morphine treatment groups (saline: p=0.0120, MEC: p=0.0411, n=8, figure 3.6) there was no difference between the time spent in drug paired chamber across the morphine treatment groups (Difference: p=0.4701, n=8).

Mecamylamine or saline pre-treatment had no effect on time spent on either side after repeated saline pairings (figure 3.7A). A one way ANOVA revealed no significant effect of treatment ($F_{(1,13)}$=0.04, p=0.8457, n=8/treatment) or test ($F_{(1,13)}$=1.16, p=0.302 n=8/treatment).

Figure 3.7B shows that treatment has a significant effect on locomotor activity as measured by distance moved. A one way ANOVA revealed a significant effect of treatment ($F_{(3,21)}$=21.73, p=<0.001, n=8/treatment. Morphine significantly increased distance moved in saline pre-treated (saline and saline: 3382±307cm vs saline and morphine 13684±1475cm p=0.001, n= 7-9) and mecamylamine pre-treated (mecamylamine and saline 4005±249cm vs mecamylamine and morphine 12756±1273cm, p=0.002). There were no differences across the saline treatment groups (saline + saline vs mecamylamine + saline: p=0.448; saline + morphine vs mecamylamine + morphine: p=0.448).

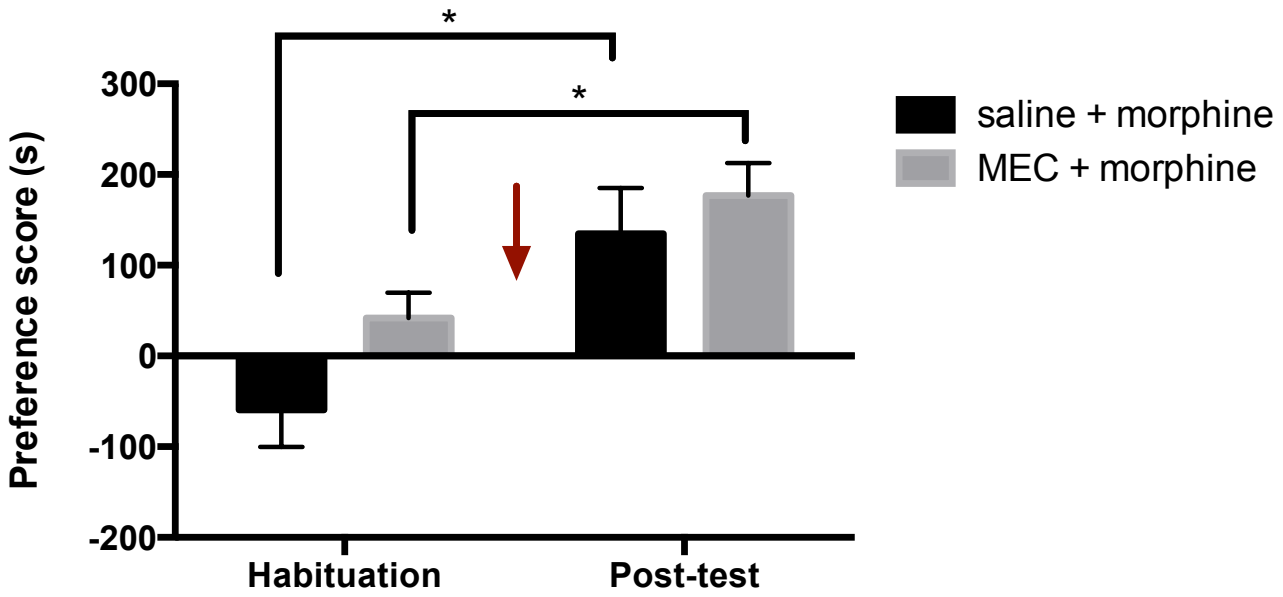


Figure 3-6 The effect of mecamylamine on the acquisition of morphine-CPP.
The effect of 1mg/kg mecamylamine s.c (red arrow) was used to determine the effect on the acquisition of morphine-CPP (data shown as mean ±SEM). Mice were randomised into one of four treatment groups: mecamylamine and morphine, mecamylamine and saline, saline and morphine or saline and saline. Mice were given either saline (10ml/kg, s.c) or mecamylamine (1mg/kg, s.c) 20 minutes prior to the morphine or saline conditioning dose daily for 4 days. Pairwise comparisons revealed no significant difference in the time spent in the drug paired side in either treatment (p=0.35).

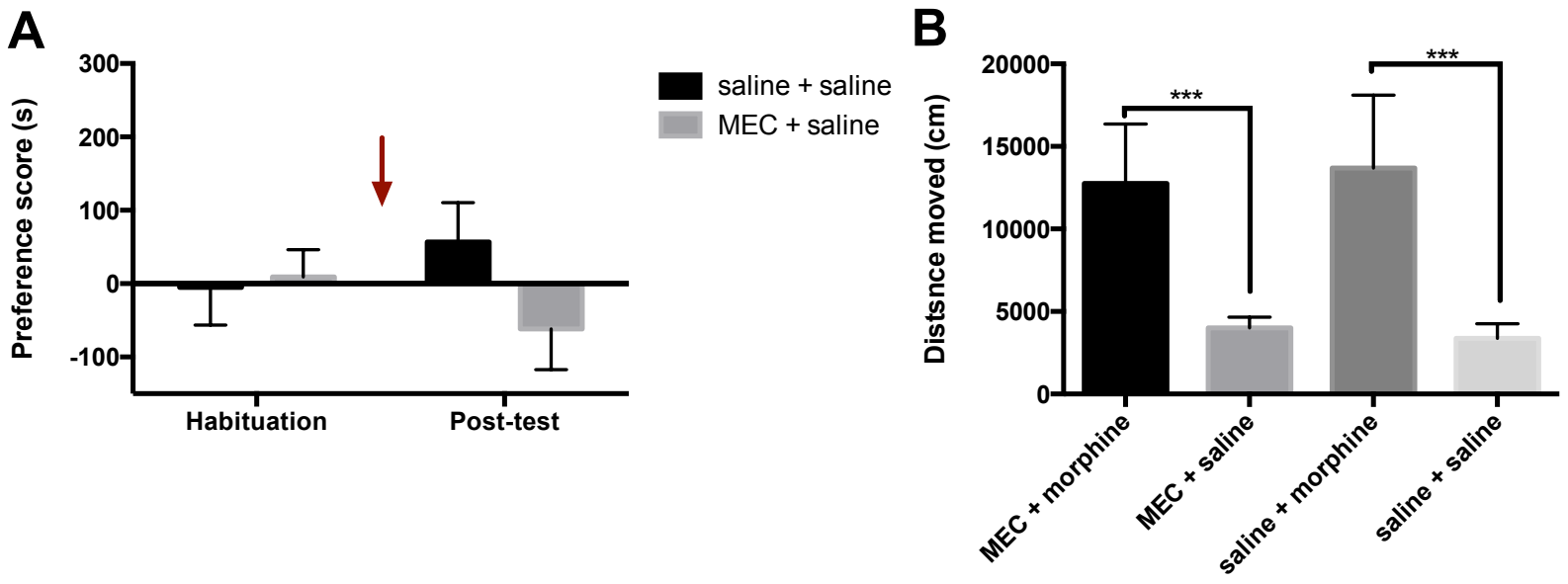


Figure 3-7 Mecamylamine has no effect on saline-CPP or distance moved. All data shown as SEM.
A) Mecamylamine pre-treatment (arrow) had no effect on time spent in drug-paired side (data shown as mean ±SEM). B) There were no significant effects of pre-treatments (Saline and saline vs mecamylamine and saline: p=0.448; saline and morphine vs mecamylamine and morphine: p=0.448) on distance moved.

The effect of mecamylamine on morphine primed reinstatement to morphine-CPP

To test whether mecamylamine resembled MLA in having an effect on the reinstatement of morphine-CPP by morphine drug priming, mecamylamine (1mg/kg, s.c) or saline was given 20 minutes prior to the morphine priming dose. Figure 3.8 shows the effect of mecamylamine on the reinstatement of morphine-CPP. A one-way ANOVA with repeated measures revealed no significance in the effect of treatment ($F_{(1,11)}$ = 2.86, p=0.119) but a significant effect of test ($F_{(3,48)}$ = 7.90, p=<0.001, n=6-12/treatment group), showing an effect of morphine conditioning but not of mecamylamine pre-treatment.

There was no significant effect of treatment on locomotion (Figure 3.8B). Animals pre-treated with mecamylamine before the morphine dose moved 8722.3±760.5cm during the reinstatement trial and animals treated with saline 20 minutes before the morphine dose moved 9059.7±1229.5cm (p=0.809).

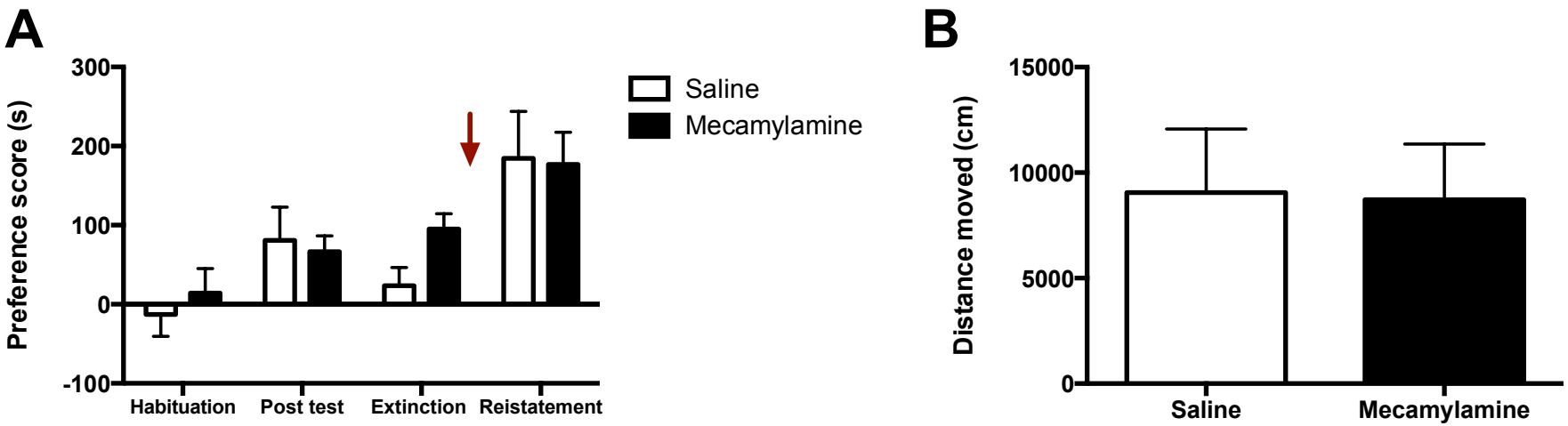


Figure 3-8 The effect of mecamylamine on morphine-CPP. All data shown as SEM.
Mecamylamine (1mg/kg, s.c) or saline was dosed 20 minutes prior (arrow) to the morphine priming dose. (data shown as mean ±SEM. A) Mecamylmine had no effect on morphine primed reinstatement. An ANOVA with repeated measures revealed no significance in the effect of treatment ($F_{(1,11)} = 2.86$, p=0.119) but a significant effect of test ($F_{(3,48)} = 7.90$, p=<0.001, n=6-12/treatment group), showing an effect of morphine conditioning but not of mecamylamine pre-treatment. B) Mecamylamine priming had no effect on distance moved after morphine treatment. Animals pre-treated with mecamylamine before the morphine dose moved 8722.3±760.5cm during the reinstatement trial and animals treated with saline 20 minutes before the morphine dose moved 9059.7±1229.5cm (p=0.809).

3.4 Discussion

The data here show that MLA, an α7 nAChR antagonist, specifically reduces reinstatement to morphine-CPP in C57BL/6Jc mice but has no effect on the acquisition, expression or reconsolidation. Furthermore, this effect of MLA was not replicated by mecamylamine, consistent with a specific role for α7 nAChRs on the reinstatement of morphine CPP.

Critique of methodology

CPP is a useful tool in investigating the associations made between a reward and a contextual cue, a factor that is thought to be increasingly important in maintaining abstinence in drug addicts. The protocol used in this chapter was validated as in Appendix A. Our data set shows increased variance in CPP reinstatement in comparison to that seen in acquisition, and this is well documented in the literature (Do Ribeiro Couto *et al.*, 2005; Shoblock *et al.*, 2005). Currently understanding of this phenomenon is limited, but there is evidence emerging in rodents and non-human primates that define a clear role for social hierarchy in addiction-related behaviours (Morgan *et al.*, 2002; Maldonado *et al.*, 2007). It has been suggested that social hierarchy can impact on reward seeking (Schenk *et al.*, 1987; Lesage *et al.*, 1999; Morgan *et al.*, 2002). However data shown in Appendix B shows no significant effect of social hierarchy on either the acquisition of morphine CPP or reinstatement (see Appendix B).

The doses of nicotinic agents reported here are in the range used previously (Chilton *et al.*, 2004; Feng *et al.*, 2011). MLA has well documented antagonistic action at α7 binding at the α-bungarotoxin binding site (Ward *et al.*, 1990) which is known to reside primarily in α7 receptors (Whiteaker *et al.*, 2000). It is important to highlight the possibility of non-specific effects of systemic MLA at high doses and there is evidence that suggests MLA may also act at α3 and α6β2 binding sites (Mogg *et al*, 2002), present mainly in catecholaminergic areas (Le Novère *et al.*, 1996). However the concentrations used in this chapter were under the nanomolar range, and were low enough not to cause any locomotor deficits or non-selective effects previously described (Chilton *et al.*, 2004; Tinsley *et al.*, 2011). The methodology used here

allowed a 20-minute pre-treatment time, to ensure sufficient MLA concentrations in the brain during the morphine dose and behavioural test. This was based on a study done by Nirogi *et al* (2012) that found peak plasma concentrations after 30 minutes post an intravenous dose (1mg/kg) or post oral dose (3mg/kg) in male Wistar rats.

MLA causes a significant reduction in reinstatement but has no effect on expression, reconsolidation or expression of morphine CPP.

Nicotinic effects have previously been reported on morphine induced withdrawal (Ise *et al.*, 2000), locomotion (Biala & Weglinska, 2004; Vihavainen *et al.*, 2006) memory impairment (Ahmadi *et al.*, 2007) and antinociception (Suh *et al.*, 1996). More recently nicotinic receptors have been implicated in the control of morphine and as well as other reward learning (Feng *et al*, 2011; Rezayof *et al*, 2006; for review see Rahman *et al.*, 2015). The primary finding of this chapter that α7 antagonism inhibits reinstatement to morphine-CPP in C57BL/6J mice but has no effect on the acquisition, expression or reconsolidation extending the findings of the same dose in Balb/c mice (Feng *et al.*, 2011).

A nAChR effect has previously been reported on memory reconsolidation but these studies are limited to stress and emotional learning rather than that to reward-related learning (for review see Blake *et al.*, 2014). If choline, an α7 agonist, is given in the mouse hippocampus immediately after training, memory of the inhibitory avoidance task (a fear based learning task) is improved and the opposite was seen with MLA (Boccia *et al.*, 2010). However this is likely to involve a different circuitry, predominately the amygdala, rather than the circuitry involved in motivational learning involved in the task in this chapter. Tinsley *et al* (2011) have shown that nicotinic antagonism doesn't impair memory after 20 minutes but does after 24hours in a model of object recognition. Although the study gave the MLA prior to the recognition test session and not after it cannot be ruled out that it may be affecting consolidation rather than acquisition mechanisms. This effect is likely to involve the entorhinal cortex, which again is not directly involved in CPP.

Mecamylamine has no effect on the acquisition or reinstatement of morphine CPP

Data shown in this chapter also shows that systemic mecamylamine (1mg/kg, s.c) had no effect on the acquisition or reinstatement of CPP. However Glick *et al* (2002) have shown that mecamylamine significantly reduced morphine self-administration, which models a different aspect of drug reward than CPP as it is a more robust model of volitional drug taking (Glick *et al.*, 2002). However, mecamylamine has been found to inhibit acquisition to morphine CPP (Zarrindast *et al.*, 2003) and bilateral injections of mecamylamine into the hippocampus (Rezayof *et al.*, 2006) and VTA (Rezayof *et al.*, 2008) dose dependently decreased morphine induced place preference. These previously-published studies differ from the experiments done in this chapter in three ways: the subjects were Wistar rats, they under went 6 conditioning trials rather than 4, and the dose was delivered intra-cranially into the hippocampus. It may be the case that the effects of mecamylamine are lost with systemic administration, as changes in bioavailability may effect the specificity if the drug.

Mecamylamine is a non-competitive nicotinic antagonist (Stone *et al.*, 1956; Varanda *et al.*, 1985; Bertrand *et al.*, 1990; Francis & Papke, 1996) that interacts with the open ion channel of the nicotinic receptor and does not bind to the agonist activation site (Banerjee *et al.*, 1990). It has been reported that mecamylamine has preferential affinity for α3β4 receptors versus other nicotinic receptors, for example α4β2 (Papke *et al.*, 2001) and is generally considered to be weaker at α7 (Albuquerque *et al.*, 2009). Therefore this might explain why here we found no effect on reinstatement or acquisition in a model very similar to Feng *et al* (2011) where they found that DHβE, a selective α4β2* antagonist inhibit reinstatement to morphine CPP. One possible explanation for why mecamylamine had no effect on morphine reinstatement or acquisition in this chapter is due to its lack of specificity to one subtype. For example in xenopus oocytes, 3μM mecamylamine produces only 50% inhibition of α4β2, α2β4, and α7 compared to a 90% inhibition at α3β2 and α3β4 (Chavez-Noriega *et al.*, 1997) However, this is from an *in vitro* binding assay and is not necessarily representative of the complex interactions *in vivo*. DHβE is a competitive antagonist which preferentially binds to β2 containing subunits and has

specific binding to α4β2 at sub-micromolar affinity but also has affinity at α3β4 and α7 although at 10-50 times lower affinity (Gotti *et al.*, 2006a). However α4β2 nAChRs have also been reported to have a role in mediating responses to cocaine (Champtiaux *et al.*, 2006), self-stimulation reward (Yeomans & Baptista, 1997) but not alcohol (Lê *et al.*, 2000; Chatterjee *et al.*, 2011).

There are also groups that have argued a hypothesised increase in acetylcholine transmission, induced by systemic administration of acetylcholinesterase inhibitors, reduces reinstatement of morphine-CPP (Gawel *et al.*, 2014**),** and volitional drug taking in heroin-induced self-administration (Zhou *et al.*, 2007). However the authors themselves point out that the effect is through a muscarinic rather than nicotinic mechanism (Zhou *et al*, 2007) and the increase in the rate of self-administration they saw with scopolamine, a muscarinic antagonist, may have been to overcome the reduced rewarding properties of each heroin injection, rather than an increase in the rewarding value of heroin. Critically, as an increase in acetylcholine wasn't actually measured in these studies it is unclear what the effect of the drug is and galantamine is also reported to act as a weak α7 potentiator (Dajas-Bailador & Wonnacott, 2004).

In an experiment conducted to investigate the effect of increased acetylcholine signalling, PNU-120596 (PAM) an allosteric positive modulator at the α7 nAChR, was used to investigate the role of endogenous acetylcholine without the complications of desensitisation. As a significant reduction in reinstatement to morphine CPP was seen with MLA pre-treatment, it was hypothesised that potentiating the receptor would increase reinstatement but have no effect on acquisition. As hypothesised, there was no effect of α7 potentiation, with PNU 120596 (PAM), on the acquisition of morphine- CPP (see figure 3.9).

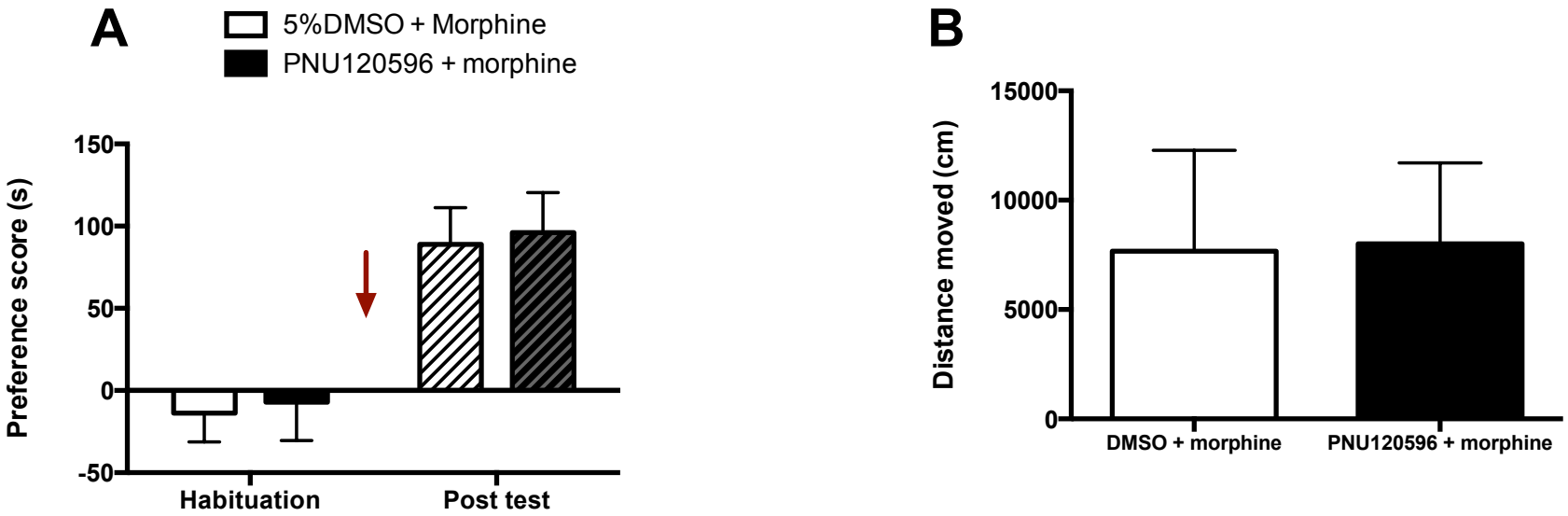


Figure 3-9 The effect of PNU 120596 on acquisition of morphine CPP.
A) To investigate the role of endogenous acetylcholine on the acquisition of CPP, we investigated whether the specific α7 nAChR positive allosteric modulator, PNU 120596 can affect acquisition of morphine-induced CPP (data shown as mean ±SEM). After habituation, animals were randomly allocated to one of two treatment groups. Either saline (10ml/kg, i.p.) or PNU 120596 (1 mg/kg, s.c.) immediately prior to morphine (10mg/kg, i.p) before each conditioning trial. A repeated measures ANOVA shows no significant effect of treatment ($F(1,16)=0.75$, $p=0.398$) but a significant effect of test ($F(1,22)=17.11$, $p=<0.001$). *Post hoc* analysis showed there was no significant difference between the PNU 1 pretreated and the saline pretreated groups ($n=16$, $p=0.595$). B) PNU120596 had no effect on distance moved moved (un-paired t-test, $p=0.8459$).

The effect of the potentiator (PAM) alongside a selective α7 nAChR agonist, PNU 282 987 (PNU) were also investigated on the reinstatement of morphine-CPP. Unfortunately, preliminary data for reinstatement (figure 3.10) of morphine-CPP was not significant, preventing assessment of drug treatments, neither of which appear to have any significant affect versus saline or morphine-treated groups. PAM alone appeared to slightly reduce drug-primed reinstatement to morphine CPP while combined treatment of the agonist and PAM slightly potentiated drug-primed reinstatement to morphine-CPP. There was no difference in the distance moved after any of the drug treatments.

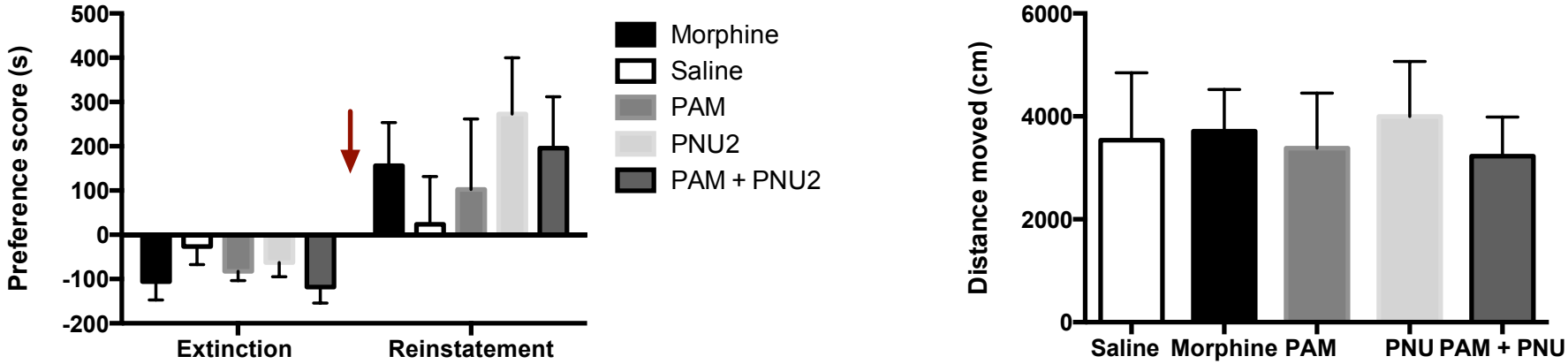


Figure 3-10 The effect of PNU-120596 and PNU282987 on reinstatement of morphine-CPP.
Preliminary data for the effect of PAM and PNU (red arrow) on reinstatement of morphine-CPP was not significant, preventing assessment of drug treatments (data shown as mean ±SEM).

Potential mechanisms for the reduction in morphine induced reinstatement seen after α7 antagonism

It is reported that learning and memory is involved in the development of opiate addiction (White, 1996; Nestler, 2001) and in animal models it has been shown that glutamate associated plasticity is required for conditioning environmental stimuli in CPP (Harris *et al.*, 2004). With this in mind there are two possible explanations for the reduction in reinstatement seen in the present study: either as an enhancement of extinction via LTP, or as a reduction in LTP and a facilitation of LTD consequently disrupting recall at reinstatement. The α7 subtype is of particular interest because it is also permeable to Ca^{2+} ions (Séguéla *et al.*, 1993; Fucile *et al.*, 2005) which links it to secondary messenger pathways hypothesised to be involved in learning and memory (Nestler, 2002; Bitner *et al.*, 2007). There is considerable evidence that α7 receptors modulate hippocampal LTP, and evidence from our laboratory has shown that MLA can reduced evoked LTP in the mPFC in a brain slice preparation (Udakis *et al.*, 2013) as well as others (Gu *et al.*, 2012; Cheng & Yakel, 2015). There is also evidence that α7 nAChRs are activated during LTD inducing stimulation to suppress LTD formation at CA3-CA1 synapse in the hippocampus (Nakauchi & Sumikawa, 2014).

3.5 Conclusions

Data in this chapter has revealed:

1) Systemic MLA (4mg,kg, s.c) specifically blocks drug primed reinstatement to morphine-CPP
2) Systemic MLA (4mg/kg, s.c.) has no effect on the reconsolidation, expression or acquisition of morphine-CPP
3) Mecamyalmine (1mg/kg, s.c) has no effect on the acquisition or reinstatement of morphine-CPP.

This suggests that α7 nAChRs have a specific role in the reinstatement to morphine-CPP. From the findings in this chapter it could be hypothesised that antagonising α7 nAChR consequently blocks endogenous acetylcholine action, which reduces the effect of the receptors in modulating morphine-CPP plasticity. The loci and mechanism for this effect will be considered in the following chapter.

CHAPTER 4 QUANTIFICATION OF [^{3}H]AMPA AND [^{3}H]MK801 BINDING SITES IN BRAIN REGIONS AFTER MORPHINE CPP

4.1 Glutamate receptors and their role in long-term potentiation in reward learning

Glutamate receptors and their role in long-term potentiation in reward learning

All drugs of abuse activate the mesolimbic dopaminergic system and repeated exposure leads to progressively stable molecular and cellular changes (Nestler, 2002). Processes involved in memory and learning are particularly involved in the conditioned aspects of reward such as the association made between the drug and environmental cues that trigger relapse. Long-term potentiation (LTP) and long term depression (LTD) are thought to occur in glutamatergic neurons in brain regions in the mesolimbic system (Nicola *et al.*, 2000; Thomas & Malenka, 2003). NMDA receptors have been shown to be critical to LTP induction in most brain regions studied, although there is evidence for some NMDA-independent LTP through calcium channels (Johnston *et al.*, 1992). Furthermore the key manifestations of LTP is an increase in postsynaptic density of AMPA receptors.

Ionotropic glutamate receptors and their distribution in the brain

Ionotropic glutamate receptors mediate excitatory neurotransmission within the central nervous system. There are two pharmacologically different families of glutamate receptors relevant to plasticity: AMPA and NMDA receptors. The highly dynamic expression of theses receptors is well characterised in the rodent brain (Blackstone *et al.*, 1992; Sucher *et al.*, 1996; Ozawa, 1998; Borges & Dingledine, 1998; Myers *et al.*, 1999). AMPA receptors are distributed ubiquitously throughout the CNS although there are some regional difference that have been demonstrated by [^{3}H]AMPA binding (Monaghan *et al.*, 1984b; Olsen *et al.*, 1987; Insel *et al.*, 1990). These studies revealed high levels of binding in the hippocampus, with higher densities in the CA1 than CA3, and in the pyramidal cell layer than the stratum radiatum and stratum oriens. High levels are also found in the molecular layer of the dentate gyrus and the superficial layer of the cerebral cortex. The deeper layer cortex and the caudate putamen have intermediate binding, whereas the diencephalon, midbrain and brain stem have lower binding still (Monaghan *et al*, 1984; Olsen *et al* 1987). Determining the location of NMDA receptors utilised a number of

radioligands including those that bind to NMDA receptors specifically [^{3}H]TCP, [^{3}H]CPP and [^{3}H]5-methyl-10,11-dihydro-5Hdibenzo[a,d]cyclohepten-5, 10-imine ([^{3}H]MK801) (Monaghan *et al.*, 1984a, 1989). These studies revealed binding throughout the brain but the highest binding was seen in the CA1 of the hippocampus, and in the forebrain.

The role of glutamate receptors in reward

Elevated dopamine transmission was thought to be the primary mediator of addictive behaviours, but, as discussed in Chapter 1, there is accumulating evidence that suggests that glutamate also plays a key role in drug addiction. Activity dependent changes in the strength of glutamatergic synapses has been shown to be fundamental for memory and learning (Pastalkova *et al.*, 2006), and is also thought to be involved in the learned associations essential for the formation and relapse of addiction (Daglish *et al.*, 2001). CPP and other animal models relevant to addiction have shown that exposure to drug-paired environments can lead to changes in glutamatergic signalling (Xia *et al.*, 2011; Portugal *et al.*, 2014) and these activity driven changes in different brain regions have been shown to contribute to different aspects of addiction, such as acquisition, withdrawal, and relapse.

Glutamate receptors have been shown to be necessary for CPP, and NMDA receptors are thought to be critical in the formation of LTP. It has been demonstrated that blockade of NMDARs with AP-5 prevents acquisition but not maintenance of both Pavlovian and operant conditioning for food (Zellner *et al.*, 2009; Ranaldi *et al.*, 2011). Systemic NMDA alone has been shown to produce CPP (Panos *et al.*, 1999) and the administration of NMDAR antagonists can block the acquisition (Rezayof *et al,* 2007), expression (Rezayof *et al.*, 2007; Li *et al.*, 2011) and reinstatement of morphine CPP (Ribeiro Do Couto *et al.*, 2005a; Ma *et al.*, 2007). The NMDAR antagonists, MK-801 and memantine, blocked the effects of morphine priming and cocaine priming-induced reinstatement of CPP (Tzschentke & Schmidt, 2003; Aguilar *et al.*, 2009). It is thought that this inhibition of reinstatement was due to the interference of NMDA antagonists with the capacity of morphine to remind the animal of the associations learned during the conditioning (Ribeiro Do Couto *et al.*, 2005a). NMDARs have been shown to regulate the magnitude of phasic DA release

to reward-associated cues (Sombers *et al.*, 2009; Zweifel *et al.*, 2009) and to alter cue-dependent reward learning (Zweifek *et al*, 2009). Glutamate receptors are also thought to play a role in reconsolidation of a drug-paired memory. For example if memantine was given during forced extinction of CPP it blocked the subsequent reinstatement. During extinction not only is the drug associated memory retrieved but a novel association is made (Ribeiro Do Couto *et al.*, 2005a; Popik *et al.*, 2006).

Methods to measure changes in glutamate receptor number and function after *in vivo* treatment.

One of the key manifestations of LTP is an increase in postsynaptic density of AMPA receptors, and there are many methods to investigate these changes. As discussed above, autoradiography has proved imperative to the discovery and exploration of the distribution of these receptors in the mammalian brain (Blackstone *et al.*, 1992; Sucher *et al.*, 1996; Ozawa, 1998; Borges & Dingledine, 1998; Myers *et al.*, 1999). The use of radioligands to determine tissue distributions of glutamate receptors has also been used extensively to explore changes after treatment *in vivo* (Yoo *et al.*, 2006). Subtype specific ligands can be labelled with ^{3}H (tritium) or ^{125}I, then incubated with brain slices, which are then apposed to x-ray sensitive film to obtain an autoradiographical image that can be analysed for density of binding as a measure of receptor expression. This technique allows high spatial resolution and allows whole brain mapping for changes in more than one receptor subtype from one behavioural experiment with low numbers of animals. This anatomical precision of this technique is invaluable when investigating the effects of a drug acting within a unknown region.

Another technique used extensively for the exploration of excitatory synapses is brain slice electrophysiology. This technique allows the exploration of functional receptors with high experimental control of the brain slice physiology, which allows manipulations to be made under certain conditions to mimic *in vivo* conditions (for review see Nauen, 2011). It maintains greater structural integrity than cell cultures or tissue homogenates and it is thought to bridge a gap between *in vivo* and in *vitro* without the cost of *in vivo* studies (Schurr, 1981). The number and ratios of NMDA and AMPA receptors can be investigated using whole cell patch clamp, as the two

receptors have distinct kinetics (AMPAτ=2-7ms; NMDAτ=50-100ms). This has been used in our laboratory to explore changes in glutamate receptors after morphine place preference (Rigby, 2012). As well as patch clamp recordings, extracellular recordings are also useful to explore changes in glutamate function. Field recordings measure changes in field excitatory postsynaptic potentials (EPSP) through evoked responses by stimulating with a small bipolar electrode, and can be used to measure pre-synaptic or postsynaptic events, either an increase in transmitter release probability from the nerve terminal or through changes in postsynaptic receptors.

Changes in receptor number can also be investigated using western blotting, which is typically used for confirming if a particular protein is in a sample and can be used, however less effectively, for the semi-quantification of protein levels. Samples such as lysed cultures or whole tissue can be run on a gel via electrophoresis, this is then transferred on to a protein sensitive membrane, such as polyvinylidene difluoride (PVDF), blocked to ensure exclusion of non-specific binding, and exposed to antibodies conjugated with either chemiluminescent enzymes or in some cases a fluorescent tag and traditionally digitization via light-sensitive X-ray films. A primary issue with western blotting is the method of translating expression bands to data that can be statistically analysed. The technique is still best utilised to identify the presence/or absence of a particular protein sample rather than to quantify protein.

Western blot can be used successfully to examine modifications of proteins such as dimerization (through changes in molecular weight, and phosphorylation, using antibodies targeted at particular phosphorylation sites. Consequently, western blotting is a powerful tool when used in-conjunction with other techniques such as subcellular fractionation where cellular compartments are sequentially extracted by incubating cells with a number of buffers to separate the cytoplasmic fraction, the membrane fraction, and the nuclear fraction. This gives higher spatial resolution, and furthermore techniques to separate pre and post-synaptic boutons have been developed to identify proteins present in the synaptic cleft (Fabian-Fine, *et al* 2000). As the trafficking of receptors between intracellular and cell surface regions of a cell is thought to be involved in synaptic plasticity other methods have been developed to measure this after *in vivo* treatment. The cell surface-expressed receptors can be

covalently cross-linked to nearby proteins using a membrane impermeable cross-linker, such as bis(sulfosuccinimidyl)suberate (BS^3). Therefore the molecular weight of these cell surface expressed receptors is altered leaving intracellular receptors unchanged, allowing the separation of these two pools of receptors via western blotting (Boudreau *et al.*, 2012). However this technique still relies on the quantification of the subsequent bands therefore it is best utilised in conjunction with other techniques.

Changes in surface expression of glutamate receptors

Using the techniques mentioned above much evidence suggests that repeated drug treatment can lead to glutamatergic changes in several brain regions. In particular hippocampal glutamatergic signalling may be important for changes in LTP that occur at different stages of morphine-CPP. It has been demonstrated that morphine CPP, extinction of morphine CPP are all associated with robust changes in hippocampal synaptic plasticity. Portugal *et al* (2014) showed that expression of morphine CPP is associated with an increase in basal synaptic transmission, impaired hippocampal LTP and increased synaptic expression of the GluN1 and GluN2b NMDAR subunits. These changes in NMDAR expression and synaptic plasticity were not observed when morphine treatment was not associated with a specific context. After extinction of morphine CPP, hippocampal LTP was impaired and synaptic GluN2a and GluN2b expression was further increased. Increases in GluN1 were also seen after stress and drug administration in the ventral tegmental area (Fitzgerald *et al*, 1996). Caffino *et al* (2014) found increased levels of both AMPA and NMDA receptors in the post-synaptic density fraction (PSD) but not in the total homogenate of the hippocampus in animals given contextual cocaine, and extinction training abolished this effect. Extinction of morphine-dependent conditioned behaviour is associated with an increased phosphorylation of the GluR1 subunit of the AMPA receptor at hippocampal slices (Billa *et al*, 2009).

Changes in glutamate receptors have been reported to be altered after reinstatement to rewards. Enhanced receptor AMPA mediated glutamate transmission has been shown to play a role in reinstatement of cocaine seeking (Kalivas *et al.*, 2005; Schmidt *et al.*, 2005b)**;** increases in GluR2 containing AMPA receptors are reported

after reinstatement of cocaine in the accumbens (Famous *et al.*, 2008) and medial prefrontal cortex (Park *et al.*, 2002). Increased AMPAR expression is also reported in reinstatement to amphetamine seeking (Cruz *et al.*, 2008). Importantly changes in glutamate receptor expression have also been shown after reinstatement to morphine place preference. Increases in GluN1 and GluN2b NMDA subunits have been reported after morphine reinstatement (Portugal *et al*, 2014).

Addiction related behaviours have been shown to be dependent on learning and memory and studies discussed above suggest a role for both NMDA and AMPA receptors in drug addiction. Evidence presented in previous chapters show that nicotinic antagonists and agonists can alter LTP and evidence suggests they may be particularly important in the formation of the association made between the reward and the environment. Receptor autoradiography provides an extremely efficient way of looking for changes in glutamate receptors, following behavioural training, within the whole mouse brain and requires relatively low animal numbers.

4.2 Aims of chapter

The aim of the work described in this chapter was to determine if reinstatement of morphine-CPP altered the distribution of NMDA and AMPA receptors in the mouse brain, and if MLA pre-treatment prevented any changes. To do this [^{3}H]MK-801 and [^{3}H]AMPA binding was monitored throughout the brain, using quantitative autoradiography, following reinstatement of morphine- CPP, with and without MLA pre-treatment.

4.3 Results

In vivo treatments for autoradiography binding experiments

24 mice underwent morphine-CPP, extinction training, and morphine or saline reinstatement. Mice were either pre-treated with MLA or saline. The treatment groups were as follows:

Pre-treatment	Reinstatement treatment	n
Saline	Saline	6
Saline	Morphine	6
MLA	Saline	6
MLA	Morphine	6

Immediately after the reinstatement session animals were sacrificed, the brains were removed and frozen in isopentane over dry ice, and snap frozen in liquid nitrogen. These brains were stored at -80°C until they were sectioned on a cryostat and consecutive sections were taken for specific and non-specific binding for each ligand.

Quantitative NMDA receptor autoradiography

[^{3}H]MK-801 autoradiography was performed in coronal slices taken at bregma 1.94mm, 1.42mm, -1.22mm, -3.08mm, refer to methods section 2.3. Representative autoradiograms of NMDA binding sites from brain sections taken from mice treated with either MLA (4mg/kg, s.c) or saline (1ml/kg, s.c) 20 minutes prior to their morphine dose (shown in red) are shown in figure 4.1. Quantitative autoradiographic analysis of all structures were carried out by reference to the mouse brain atlas of Franklin and Paxinos (1997) and binding was analysed using MCID image analyser (refer to methods section 2.3)

The mean density of specific [^{3}H]MK-801 binding in brain regions is shown for all areas analysed in table 4.1. NMDA binding was particularly high in cortical areas as well as in the hippocampus and thalamic areas. Non-specific binding was found to be homogenous and density in corresponding areas was subtracted from total binding.

A two-way ANOVA showed no effect of pre-treatment or treatment in any of the regions, showing neither reinstatement dose (morphine or saline) or pre-treatment (MLA or saline) had any effect on [^{3}H]MK-801 binding density compared to saline (see Appendix C for statistics for all regions).

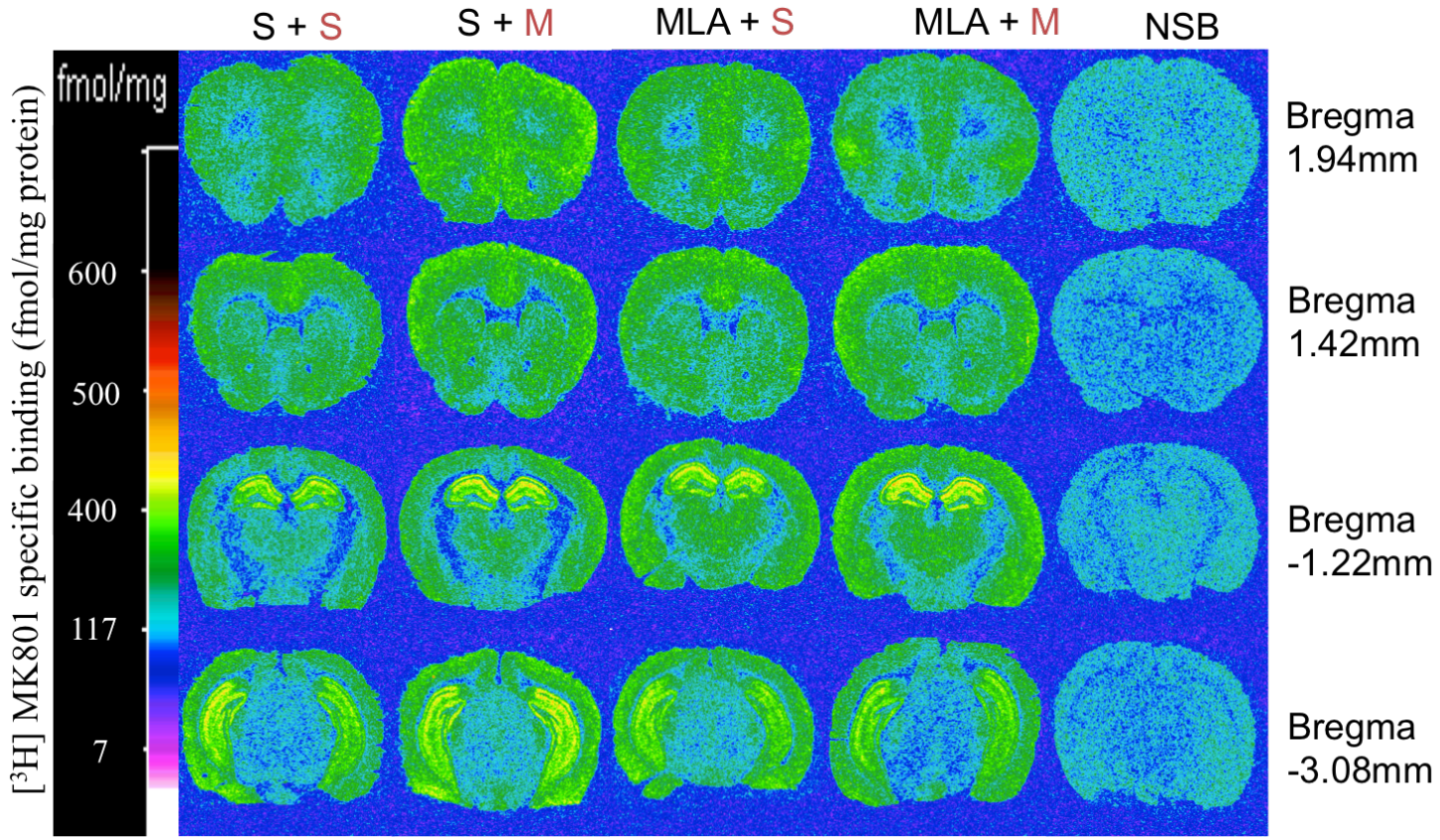


Figure 4-1 Representative autoradiograms of [^{3}H]MK-801 binding following either MLA pre-treatment or morphine reinstatement.
The panels show coronal sections cut at the level of the mPFC (bregma 1.94mm), striatum (bregma 1.42mm), the dorsal hippocampus (bregma -1.22mm) and ventral hippocampus (bregma -3.08mm). To label NMDA receptors adjacent sections were incubated for 1 hour at room temperature with 70nM [^{3}H]MK-801 alone or in the presence of 1mM MK801 to calculate non-specific binding. Sections were apposed to Kodak film for 3 weeks. The bar shows colour image density calibrated in fmol/mg of tissue equivalent.

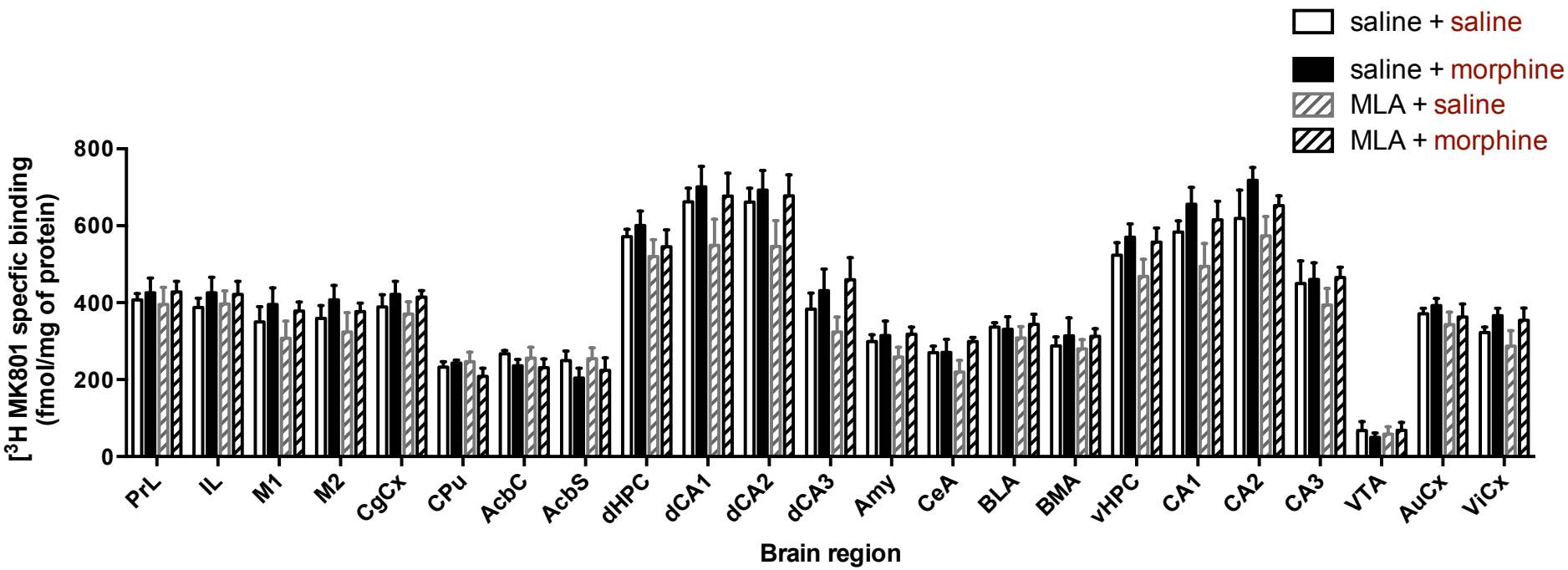


Figure 4-2 Changes in [^{3}H]MK801 binding after pre-treatment with saline or MLA prior to morphine primed reinstatement.
There were no significant changes in any of the regions quantified after any treatment (data shown as mean ±SEM). Regions quantified: Prelimbic (PrL), Infralimbic (IL), Motor cortices (M1-2), Cigulate cortex (CgCx), Caudate putamen (CPu), Accumbens shell (Acbs) and core (Acbc), dorsal hippocampus (dHPC), dorsal CA1-CA3, Amygdala (Amy), including Central (CeA), Basolateral (BLA), and Basomedial (BMA), ventral hippocampus (vHPC) including ventral CA1-3, Ventral tegmental area (VTA), Auditory cortex (AuCx) and visual cortex (ViCx).

Table 4-1 Changes in [^{3}H]MK801 binding after pre-treatment with saline or MLA prior to morphine primed reinstatement.
There were no significant changes in any of the regions quantified after any treatment (data shown as mean ±SEM). Regions quantified: Prelimbic (PrL), Infralimbic (IL), Motor cortices (M1-2), Cigulate cortex (CgCx), Caudate putamen (CPu), Accumbens shell (Acbs) and core (Acbc), dorsal hippocampus (dHPC), dorsal CA1-CA3, Amygdala (Amy), including Central (CeA), Basolateral (BLA), and Basomedial (BMA), ventral hippocampus (vHPC) including ventral CA1-3, Ventral tegmental area (VTA), Auditory cortex (AuCx) and visual cortex (ViCx).

		[3H] MK801 specific binding (fmol/mg protein)							
		Morphine Reinstatement				MLA pretreatment Reinstatement			
Bregma	Region	Saline	Morphine	Percentage change (%)	p value	Morphine	MLA	Percentage change (%)	p value
1.94mm	PrL	407.2 ±16.5	426.2 ±35.4	4.7	ns	426.2 ±35.4	428.0 ±27.4	0.4	ns
	IL	387.3 ±24.6	425.5 ±38.2	9.9	ns	425.5 ±38.2	421.9 ±33.5	-0.8	ns
	M1	350.0 ±39.7	395.6 ±40.0	13.0	ns	395.6 ±40.0	378.5 ±23.5	-4.3	ns
	M2	359.0 ±33.7	407.5 ±35.0	13.5	ns	407.5 ±35.0	377.1 ±22.2	-7.5	ns
	CgCx	388.8 ±32.3	421.7 ±31.8	8.4	ns	421.7 ±31.8	414.2 ±17.1	-1.8	ns
1.42mm	CPu	232.3 ±14.6	243.0 ±7.3	4.6	ns	243.0 ±7.3	208.4 ±21.0	-14.2	ns
	AcbC	267.3 ±8.2	235.8 ±15.8	-11.8	ns	235.8 ±15.8	231.4 ±22.6	-1.9	ns
	AcbS	249.2 ±25.0	204.0 ±24.4	-18.1	ns	204.0 ±24.4	224.3 ±32.7	10.0	ns
-1.22mm	BMA	287.7 ±23.3	314.2 ±43.3	9.2	ns	314.2 ±43.3	312.4 ±19.7	-0.6	ns
	Amy	299.3 ±17.3	314.9 ±34.6	5.2	ns	314.9 ±34.6	31.9 ±19.0	0.9	ns
	CeA	270.3 ±16.9	270.5 ±31.8	0.1	ns	270.5 ±31.8	299.0 ±11.0	10.5	ns
	BLA	336.8 ±11.2	331.1 ±29.9	-1.7	ns	331.1 ±29.9	343.5 ±26.2	3.7	ns
	dHPC	571.8 ±19.3	600.5 ±34.9	5.0	ns	600.5 ±34.9	545.8 ±43.8	-9.1	ns
	dCA1	662.3 ± 35.6	701.4 ± 53.0	5.9	ns	701.4 ± 53.0	677.4 ± 59.3	-3.4	ns
	dCA2	661.3 ± 36.5	692.5 ± 51.5	4.7	ns	692.5 ± 51.5	677.7 ± 54.7	-2.1	ns
	dCA3	383.2 ± 41.9	431.5 ± 56.1	12.6	ns	431.5 ± 56.1	459.9 ± 57.0	6.6	ns
-3.08mm	vHPC	523.3 ±33.0	570.4 ±32.5	9.0	ns	570.4 ±32.5	557.8 ±36.6	-2.2	ns
	CA1	583.6 ±29.0	656.1 ±40.9	12.4	ns	656.1 ±40.9	615.6 ±48.2	-6.2	ns
	CA2	619.0 ±73.5	718.2 ±31.3	16.0	ns	718.2 ±31.3	652.3 ±25.7	-9.2	ns
	CA3	450.0 ±58.9	460.3 ± 40.8	2.3	ns	460.3 ± 40.8	465.3 ±27.1	1.1	ns
	VTA	67.3 ±23.6	49.8 ±10.8	-26.0	ns	49.8 ±10.8	68.1 ±20.9	36.8	ns
	AuCx	371.1 ±14.7	392.3 ±17.5	5.7	ns	392.3 ±17.5	362.9 ±33.9	-7.5	ns
	ViCx	322.7 ±14.1	366.7 ±17.7	13.6	ns	366.7 ±17.7	354.3 ±32.0	-3.4	ns

Quantitative AMPA receptor autoradiography

[^{3}H]AMPA autoradiography was performed in coronal slices taken at bregma 1.94mm, 1.42mm, -1.22mm, -3.08mm. Representative autoradiograms of AMPA binding sites from brain sections taken from mice treated with either MLA (4mg/kg, s.c) or saline (1ml/kg, s.c) 20 minutes prior to their morphine dose (shown in red) are shown in figure 4.3. Quantitative autoradiographic analysis of all structures were carried out by reference to the mouse brain atlas of Franklin and Paxinos (1997) and binding was analysed using MCID image analyser (refer to methods section 2.3).

The mean density of specific [^{3}H]AMPA binding in brain regions is shown for all areas analysed in Table 4.2. AMPA binding was particularly high in cortical areas as well as the hippocampus, but very low in thalamic areas.

A two-way ANOVA showed neither reinstatement dose (morphine or saline) or pre-treatment (MLA or saline) had any effect on [^{3}H]AMPA binding density compared to saline in bregma 1.94mm or 1.42mm. However at bregma -3.08mm morphine reinstatement significantly increased [^{3}H]AMPA binding density in the ventral hippocampus compared to saline (p=0.0104, n=4-6/treatment group). There was no significant effect of pre-treatment (MLA or saline) in vHPC (p=0.07, n=4-6/treatment group). However analysis of the sub-regions of the ventral hippocampus revealed significant increases in [^{3}H]AMPA binding density after morphine reinstatement in the CA1 (p=<0.0001, n=4-6/treatment group) and the CA2 (p=0.0053, n=4-6/treatment group) and this was significantly reduced with MLA pre-treatment (CA1:p=0.027, CA2: p=0.0311, with a significant interaction: p=0.0456, n=4-6/treatment group).

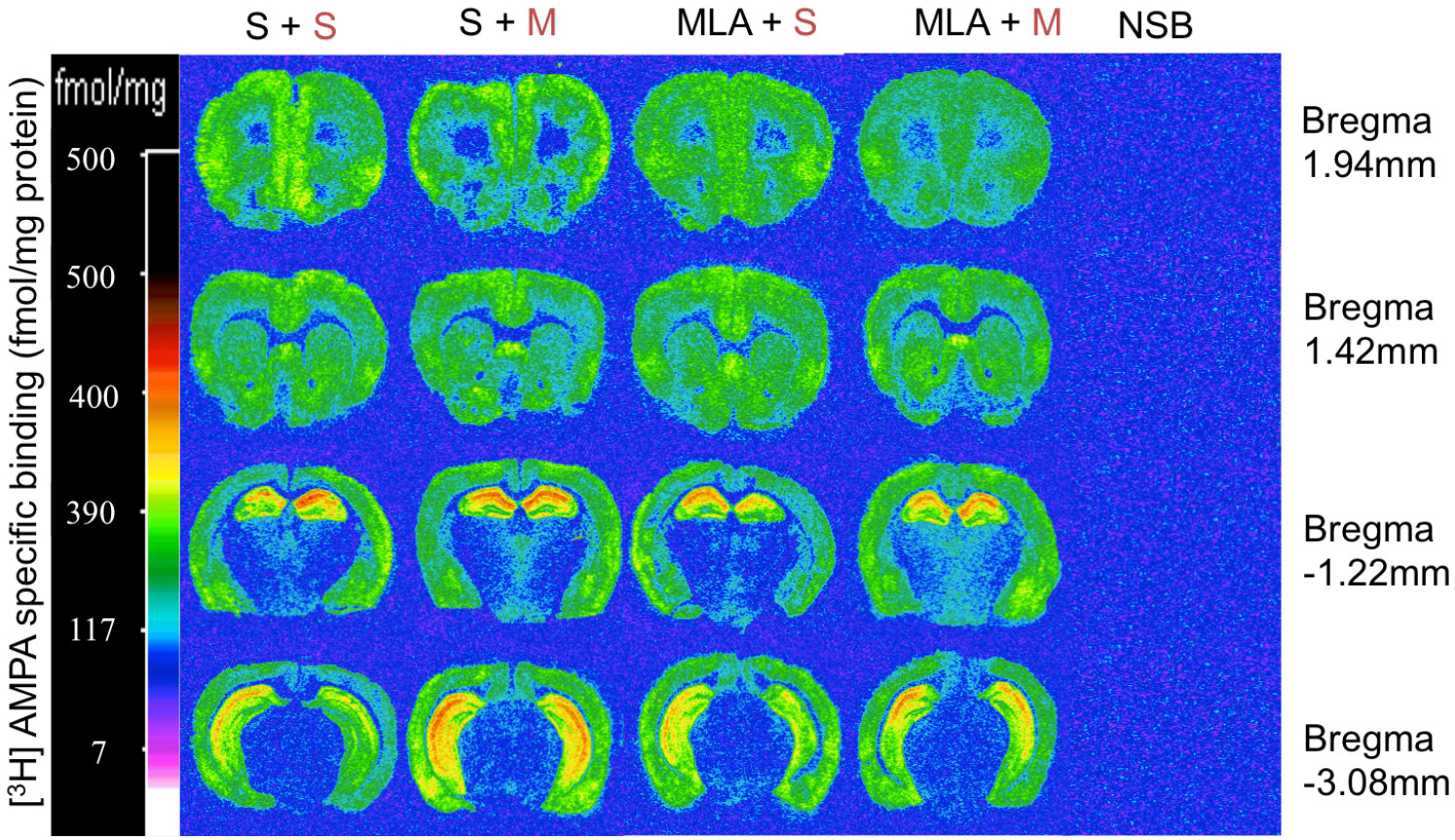


Figure 4-3 Representative [3H]AMPA binding density autoradiograms of binding following either MLA pre-treatment or morphine reinstatement.
The panels show coronal sections cut at the level of the mPFC (bregma 1.94mm), striatum (bregma 1.42mm), the dorsal hippocampus (bregma -1.22mm) and ventral hippocampus (bregma -3.08mm). To label AMPA receptors adjacent sections were incubated for 45 minutes at room temperature with 10nM [^{3}H]AMPA alone or in the presence of 0.1mM CNQX to calculate non-specific binding. Sections were apposed to Kodak film for 4 weeks. The bar shows colour image density calibrated in fmol/mg of tissue equivalent.

Table 4-2 Changes in [^{3}H]AMPA binding after pre-treatment with saline or MLA prior to morphine primed reinstatement.
There were no significant changes in: Prelimbic (PrL), Infralimbic (IL), Motor cortices (M1-2), Cigulate cortex (CgCx), Caudate putamen (CPu), Accumbens shell (Acbs) and core (Acbc), dorsal hippocampus (dHPC), dorsal CA1-CA3, Amygdala (Amy), including Central (CeA), Basolateral (BLA), and Basomedial (BMA), Ventral tegmental area (VTA), Auditory cortex (AuCx) and visual cortex (ViCx). Two-way ANOVA (n=4-6/treatment group) shows that morphine reinstatement significantly increased [^{3}H]AMPA binding in the vHPC (p=0.0104), vCA1(p=<0.0001), vCA2 (p=0.0053) and this was significantly reduced by MLA pre-treatment in the CA1 (p=0.0027), and CA2 (p=0.0311). In the CA2 there was a positive interaction between treatment and pre-treatment (p=0.0456). All data shown as mean ±SEM.

		[3H] AMPA specific binding (fmol/mg protein)							
		Morphine Reinstatement				MLA pretreatment Reinstatement			
Bregma	Region	Saline	Morphine	Percentage change (%)	p value	Morphine	MLA	Percentage change (%)	p value
1.94mm	PrL	216.9 ± 08.3	226.7 ± 23.3	4.5	ns	226.7 ± 23.3	177.9 ± 11.0	-21.5	ns
	IL	208.9 ± 17.5	199.3 ± 24.1	-4.6	ns	199.3 ± 24.1	184.1 ± 18.6	-7.6	ns
	M1	184.8 ± 07.0	204.3 ± 10.3	10.6	ns	204.3 ± 10.3	179.5 ± 19.7	-12.1	ns
	M2	202.0 ± 04.8	221.2 ± 12.1	9.5	ns	221.2 ± 12.1	194.3 ± 17.5	-12.2	ns
	CgCx	211.6 ± 03.6	228.5 ± 21.7	8.0	ns	228.5 ± 21.7	189.3 ± 14.3	-17.1	ns
1.42mm	CPu	131.1 ± 01.3	122.2 ± 7.00	-6.8	ns	122.2 ± 7.00	140.3 ± 11.1	14.8	ns
	AcbC	188.2 ± 07.2	190.5 ± 11.6	1.2	ns	190.5 ± 11.6	188.7 ± 18.8	-0.9	ns
	AcbS	168.1 ± 17.4	148.4 ± 16.5	-11.7	ns	148.4 ± 16.5	171.8 ± 18.5	15.8	ns
-1.22mm	BMA	195.1 ± 11.5	185.3 ± 5.00	-5.0	ns	185.3 ± 5.00	189.6 ± 21.3	2.3	ns
	Amy	188.8 ± 10.2	183.1 ± 6.60	-3.0	ns	183.1 ± 6.60	174.5 ± 17.1	-4.7	ns
	CeA	180.7 ± 17.2	187.6 ± 9.90	3.8	ns	187.6 ± 9.90	181.6 ± 19.9	-3.2	ns
	BLA	203.3 ± 15.1	200.5 ± 8.60	-1.4	ns	200.5 ± 8.60	193.8 ± 17.7	-3.4	ns
	dHPC	299.8 ± 12.1	320.9 ± 9.10	7.0	ns	320.9 ± 9.10	278.7 ± 19.2	-13.2	0.021
	dCA1	363.6 ± 20.5	352.3 ± 54.0	-3.1	ns	352.3 ± 54.0	360.2 ± 40.0	2.2	ns
	dCA2	351.8 ± 21.0	314.1 ± 49.1	-10.7	ns	314.1 ± 49.1	328.9 ± 29.5	4.7	ns
	dCA3	202.6 ± 20.4	160.2 ± 27.2	-20.9	ns	160.2 ± 27.2	178.0 ± 24.3	11.1	ns
-3.08mm	vHPC	275.8 ± 13.9	346.9 ± 17.4	25.8	0.010	346.9 ± 17.4	287.7 ± 15.2	-17.1	0.045
	vCA1	322.2 ± 11.2	416.3 ± 10.9	29.2	<0.001	416.3 ± 10.9	365.0 ± 19.9	-12.3	0.016
	vCA2	313.8 ± 13.2	428.5 ± 23.6	36.6	0.006	428.5 ± 23.6	323.4 ± 21.7	-24.5	0.016
	vCA3	255.8 ± 23.8	304.1 ± 20.8	18.9	ns	304.1 ± 20.8	275.6 ± 16.6	-9.4	ns
	VTA	023.2 ± 03.2	50.00 ± 11.3	115.5	ns	50.00 ± 11.3	38.1 ± 4.8	-23.8	ns
	AuCx	178.8 ± 12.5	204.8 ± 11.9	14.5	ns	204.8 ± 11.9	157.7 ± 11.0	-23.0	ns
	ViCx	162.5 ± 16.7	203.7 ± 15.3	25.4	ns	203.7 ± 15.3	165.8 ± 12.3	-18.6	ns

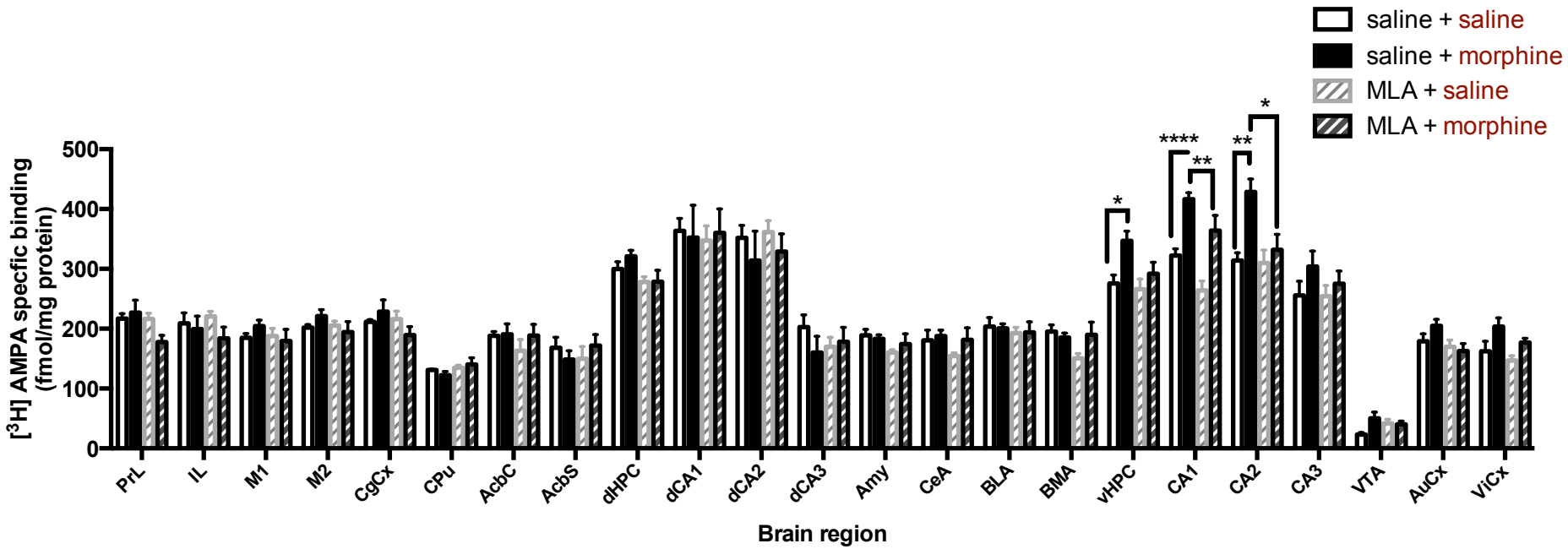


Figure 4-4 Changes in [^{3}H]AMPA binding after pre-treatment with saline or MLA prior to morphine primed reinstatement.
There were no significant changes in: Prelimbic (PrL), Infralimbic (IL), Motor cortices (M1-2), Cigulate cortex (CgCx), Caudate putamen (CPu), Accumbens shell (Acbs) and core (Acbc), dorsal hippocampus (dHPC), dorsal CA1-CA3, Amygdala (Amy), including Central (CeA), Basolateral (BLA), and Basomedial (BMA), Ventral tegmental area (VTA), Auditory cortex (AuCx) and visual cortex (ViCx). Two-way ANOVA (n=4-6/treatment group) shows that morphine reinstatement significantly increased [^{3}H]AMPA binding in the vHPC (p=0.0104), vCA1(p=<0.0001), vCA2 (p=0.0053) and this was significantly reduced by MLA pre-treatment in the CA1 (p=0.0027), and CA2 (p=0.0311). In the CA2 there was a positive interaction between treatment and pre-treatment (p=0.0456). All data shown as mean ±SEM.

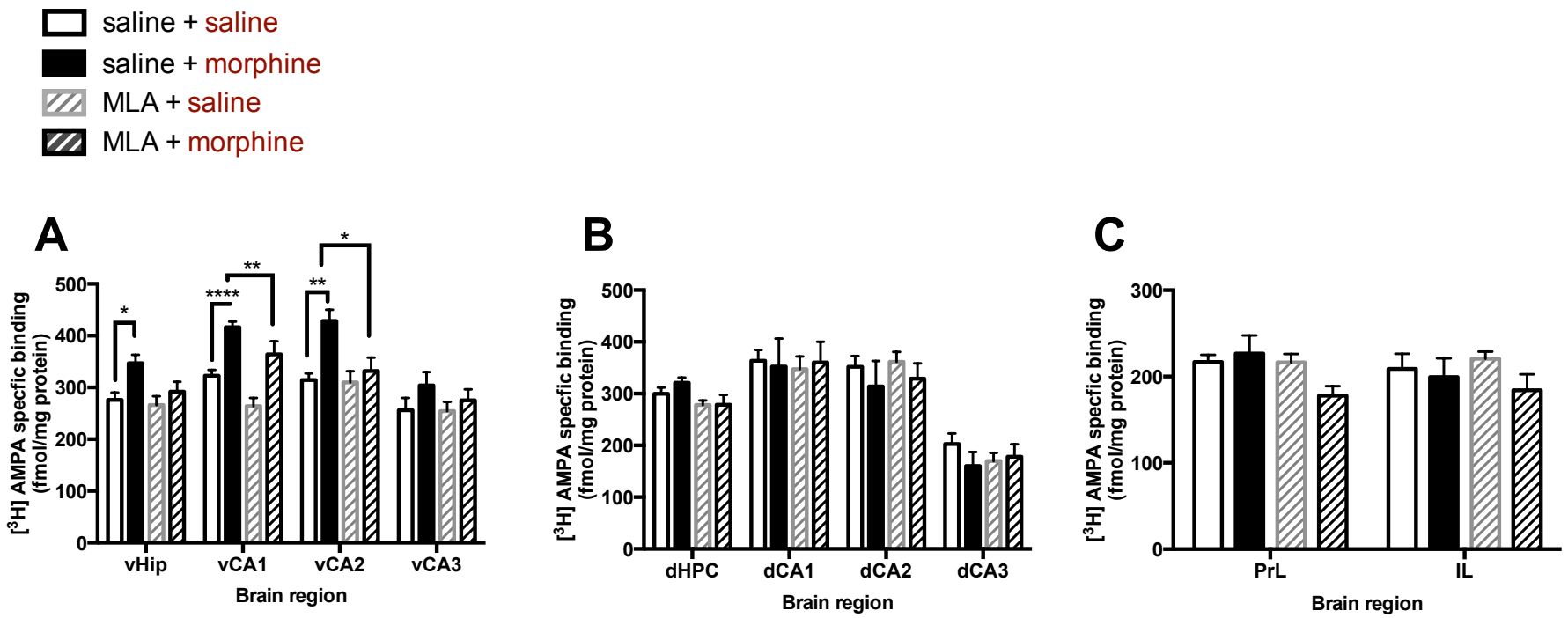


Figure 4-5 Changes in [^{3}H]AMPA binding after pre-treatment with saline or MLA prior to morphine primed reinstatement in the hippocampus and mPFC.

A) In the ventral HPC significant increases in [^{3}H]AMPA binding density in the after morphine treatment CA1 (p=<0.0001, n=4-6/treatment group) and the CA2 (p=0.0053, n=4-6/treatment group) and this was significantly reduced with MLA pre-treatment (CA1:p=0.027, CA2: p=0.0311, with a significant interaction: p=0.0456, n=4-6/treatment group). B) There was no effect of morphine reinstatement or MLA pre-treatment in the dorsal hippocampus of any of the sub regions. C) There was no effect of morphine reinstatement or MLA pre-treatment in the either region of the mPFC. All data shown as mean ±SEM.

4.4 Discussion

Autoradiography to investigate glutamate receptor binding after morphine-CPP

Quantitative receptor autoradiography of sections allows the precise location of receptors and computerised microdensitometry allows feasible quantitative determinations in tissue from one animal, forming complete brain mapping for the receptor. In this sense the technique provides a huge amount of data from a relatively small amount of animals. However, the technique lacks the resolution to investigate subtype specific changes and it is also unclear where the receptors are at a cellular level (discussed later). The qualitative and quantitative [^{3}H]MK-801 autoradiography binding revealed highest binding in the CA1 and CA2, high binding in frontal areas such as the mPFC and the cingulate cortex, as well as relevantly consistent binding throughout all other areas measured in coronal sections of C57BL/6J mice. This finding is in agreement with other studies (Monaghan *et al.*, 1984b, 1989).

The [^{3}H]AMPA binding results suggest that AMPA receptors are distributed ubiquitously throughout the CNS although there are some regional differences. The highest levels of binding were seen in the CA1 and CA2 of the hippocampus, as well as the medial prefrontal cortex and the BLA. The caudate putamen, the nucleus accumbens and areas of the visual and auditory cortex had intermediate binding, whilst midbrain areas such as the VTA had low binding. The binding pattern reported here for saline reinstated animals was comparable to previous reports in naïve rodents (Monaghan *et al.*, 1984b; Olsen *et al.*, 1987; Insel *et al.*, 1990). The data suggest that the [^{3}H]MK801 binding levels were higher than [^{3}H]AMPA levels and this seems to be as reported in the literature (Palomero-Gallagher *et al.*, 2003). However the levels of [^{3}H]AMPA binding that are reported here are lower than previously reported (Monaghan *et al.*, 1984b, 1989; Palomero-Gallagher *et al.*, 2003) suggesting a potentially low ligand concentration, however 10nM [^{3}H]AMPA is a standard protocol. These studies, cited above, were done in rats so this may account for the discrepancy with the current study.

We found lower levels of [^{3}H]-AMPA and [^{3}H]-MK801 binding in the vHPC in the saline treated mice compared to the dorsal hippocampus (although these animals

received morphine-CPP). Pandis *et al* (2006) found lower levels of mRNA, protein expression and [^{3}H]-AMPA and [^{3}H]-MK801 binding levels in the ventral hippocampus as well as longer NMDA receptor mediated excitatory postsynaptic potentials in the ventral hippocampus compared with the dorsal hippocampus via intracellular recordings.

Morphine reinstatement increased [^{3}H]AMPA binding in the ventral hippocampus, what does this mean at a subcellular level?

In animals reinstated with morphine (5mg/kg, ip) there was a significant increase in the [^{3}H]AMPA binding in the ventral CA1 (p=<0.0001) and CA2 (p=0.0053) but not the dorsal hippocampus (CA1: p=0.0589, CA2: p=0.0787). Activity dependent changes in the strength of excitatory glutamatergic synapses has been shown to be fundamental for memory and learning (Pastalkova *et al.*, 2006) and these processes are thought to be involved in the learned associations essential for the formation and relapse of addiction (Daglish *et al.*, 2001).

If this change in [^{3}H]AMPA binding seen in this chapter is to be interpreted as a change in synaptic plasticity, one must do so with caution. An increase in binding could mean an increase in cell surface expressed receptor, either from translocation from intracellular vesicles or extrasynaptic sites (Malinow *et al.*, 2000). Although there is evidence of AMPA mRNA synthesis occurring as soon as 15minutes after a stimulation (90mM KCL for 1min followed by a 15min incubation) (Orlandi *et al.*, 2011), protein synthesis and potential translocation from cell body to the synapse takes longer (although it may occur in dendritic spines). Furthermore, it has been shown that protein and RNA synthesis is required for LTP phases occurring more than an hour post intervention (Nguyen & Kandel, 1996; Ran *et al.*, 2013). It is unlikely that increased binding is an increase in *de novo* total receptors as the behaviour treatment occurred only 30 minutes prior to sacrifice. Therefore it could be hypothesised that increase in binding was either representative of either an increase in insertion (through lateral diffusion from extrasynaptic site or vesicles) or a decrease in the removal of AMPA receptors.

Autoradiography cannot distinguish synaptic from extrasynaptic locations, as they are both externally facing in the membrane and within microns of one another. Therefore, future experiments would lend themselves to the investigation of ERK activation via western blotting as an increase has been previous associated with AMPA insertion in the hippocampus (Zhu *et al.*, 2002). AMPA receptors have been shown previously to be involved in opiate induced plasticity controlling craving, withdrawal (Das *et al.*, 2008) and relapse (Cruz *et al.*, 2008; Van den Oever *et al.*, 2008; Famous *et al.*, 2008). Furthermore it seems that only context-dependent behavioural memories show increased synaptic AMPAR expression in the hippocampus (Xia *et al.*, 2011).

Modifications to the receptors themselves have been shown to increase channel open probability, increased single channel conductance (Benke *et al.*, 1998; Derkach *et al.*, 1999)**,** change affinity and modify channel kinetic properties. For example changes in AMPA channel phosphorylation can increase single channel conductance (Benke *et al.*, 1998). Receptor affinity has also been shown to be modulated by phosphorylation (Kwatra *et al.*, 1989). Therefore it could be argued that an increase in receptor affinity may also account for the increase in binding. However, Andrásfalvy & Magee (2004) showed no change in glutamate affinity was observed following tetanus induced potentiation.

Future experiments

Further experimentation is required to fully interpret these findings as presently, the data shows an increase in binding but it is unknown where these receptors are and what subunits are involved. Protein crosslinking, which has been used to investigate the location of AMPAR (Boudreau & Wolf, 2005) and protein phosphorylation states of different subunits could be examined using western blots (Xia *et al.*, 2011). Not all subunits respond to AMPA equally and it is thought that the GluR1 subtype plays a more central role in modulating synaptic plasticity due to its high permeability to calcium (Billa *et al.*, 2010). *In vivo* treatment has been shown to alter the ratios of different subtypes in a process known as subunit switching. For example repeated morphine treatment decreased surface expression of GluR1 in the mPFC without affecting levels of GluR2 (Mickiewicz & Napier, 2011) and the number of AMPA

receptors in the nucleus accumbens is increased after prolonged withdrawal from cocaine administration by addition of new AMPA receptors lacking the GluR2 subunit (Ferrario *et al.*, 2011).

Implications for stages of CPP

As discussed previously glutamatergic changes have been reported in modulating responses to drugs of abuse. It has been demonstrated that morphine CPP (Caffino *et al.*, 2014; Portugal *et al.*, 2014), as well as extinction of morphine CPP (Billa *et al.*, 2009) are both associated with robust changes in hippocampal synaptic plasticity. Others have reported changes in AMPA subunit expression after reinstatement to cocaine (Kalivas *et al.*, 2005; Schmidt *et al.*, 2005a; Famous *et al.*, 2008) and to amphetamine (Cruz *et al.*, 2008). Increases in GluN1 and GluN2b NMDA receptor subunits have been reported after morphine reinstatement (Portugal *et al*, 2014), however the data in this chapter is the first evidence of changes in [^{3}H]AMPA binding after morphine reinstatement. As the chapter only examined changes in total AMPA and NMDA rather than changes in specific subunits potential effects localised at the synapse may be diluted and that may explain why we saw no changes in [^{3}H]MK801 binding.

Previously the medial prefrontal cortex and the accumbens have been implicated as a loci for these changes (Park *et al.*, 2002; Famous *et al.*, 2008). However we see the largest percentage change from saline to morphine drug primed reinstatement in [^{3}H]NMDA binding in hippocampal regions (CA1: 12.4% CA2:16.0% change from saline). Although these are not significant, at a cell surface level significance may be present (as discussed above). The hippocampus has been implicated in CPP (Ferbinteanu & McDonald, 2001), but studies showing a change in the NMDA subunits expression in the locus coeruleus and the hypothalumus after chronic morphine treatment but not the hippocampus (Zhu *et al.*, 1999). However this was non-contingent morphine administration and the hippocampus may be more important in the contextual association made with the drug. The hippocampal NMDARs have been shown to be important in the effects of morphine dependency on spatial learning in the rat (Pourmotabbed *et al.*, 2006) and others have found that the NR2B containing NMDA receptor is important for the recall of a morphine

associated memory in the shell but not the core of the caudate putamen (Xu *et al.*, 2012). However we only saw a 4.6% increase in total NMDA binding in this area, suggesting that investigating the role of NMDA receptor subtypes would be an interesting follow up experiment.

Inhibition of α7 nAChRs prevents morphine induced increase in [^{3}H]AMPA binding

The increase in[^{3}H]AMPA binding was significantly reduced with MLA pre-treatment in the ventral hippocampus. This decrease in [^{3}H]AMPA binding can also follow the hypothesis of a block of nicotinic receptors (Hunter *et al.*, 1994; Nishizaki *et al.*, 1999; Fujii *et al.*, 1999) as, in the hippocampus activation of these receptors has been shown to facilitate the induction of LTP and mecamylamine suppresses it. To understand whether this effect is a local effect at a synaptic level in the hippocampus we need to refer back to where α7 nAChRs are present in the brain and at the synapse. α7 expression is high throughout the hippocampus (Martin & Aceto, 1981; Séguéla *et al.*, 1993). Their expression is particularly high in the CA1, CA3 and dentate gyrus (Ji & Dani, 2000; Alkondon & Albuquerque, 2001; Sharma & Vijayaraghavan, 2003; Tang *et al.*, 2011b) and the main input of cholinergic innervation is from the medial septum and diagonal band which projects through the CA1-CA3. These area are referred to as the hippocampus proper and lesions of this area have been shown to abolish morphine-CPP (Ferbinteanu & McDonald, 2001). They are present both pre-synaptically and post-synaptically at both glutamatergic and GABAergic synapses (Fabian-Fine *et al.*, 2001).

Within the hippocampus the main input of cholinergic fibres comes from the medial septum via the fimbria-fornix (Dutar *et al.*, 1995). This input into the hippocampus allows α7 receptors to modulate synaptic plasticity in a number of ways (Fujii & Sumikawa, 2001; Ji *et al.*, 2001b; McGehee, 2002a; Cobb & Davies, 2005; Maylie & Adelman, 2010). Since cholinergic receptors are present both pre- and postsynaptically at glutamatergic sites, they have the potential to coordinate pre- and postsynaptic activity to induce plasticity. When activated with ACh presynaptic nAChRs can modulate neurotransmitter release because of the cationic current through nAChRs can depolarise membranes and raise intracellular Ca^{2+} levels

directly and indirectly through intracellular stores (Dajas-Bailador *et al.*, 2002a), which triggers neurotransmitter release from the presynaptic terminal. α7 nAChRs can also presynaptically increase frequency of spontaneous EPSCs, increasing the amplitude of the synaptic event increasing in the probability of glutamate release (Pidoplichko *et al.*, 2004).

nAChRs are present on GABAergic nerve terminals in the hippocampus, indeed interneurons in the hippocampus actually express much high levels of nAChRs than pyramidal cells (Frazier *et al.*, 1998; McQuiston & Madison, 1999; Ji *et al.*, 2001b; Yakel & Shao, 2004). When ACh is applied to a CA1 interneuron, the GABAergic input to the pyramidal neurons is enhanced and this effectively blocks the induced short-term potentiation (STP) and long-term potentiation (LTP) (Ji *et al.*, 2001a) but this effect varied depending on the timing of when the STP and Ach were applied. The importance of these GABAergic projections has been emphasised, particularly in the genesis of rhythmic activity in the medial septum and hippocampus, known as theta rhythm, which is though to be important for memory formation and consolidation. Encoding is enhanced when stimuli are present during theta rhythm (Bland & Oddie, 2001; Griffin *et al.*, 2004) and activation of α7 receptors has been shown to facilitate these oscillations (Siok *et al.*, 2006). It is thought that GABA regulates overall circuit tone and thereby serves to bind or sustain an animal's motivational state until the goal object can be achieved (McFarland *et al.*, 2003). Van den Oever, *et al* (2010) have made a case for the importance of GABAergic signalling in controlling relapse to heroin. Patterns of GABAergic activity have been shown to provide spatial and temporal cues for modifying synaptic weight and therefore prompt encoding and retrieval of memory in the hippocampus (Wallenstein & Hasselmo, 1997; Paulsen & Moser, 1998; Van den Oever *et al.*, 2010).

The effect of MLA appears to be specific to reinstatement and has no effect on other stages of morphine-CPP, as shown in chapter 3. The specificity of this effect is intriguing. As mentioned previously LTP follows a series of stages after plasticity inducing activity. The degree of initial encoding, and later consolidation, are thought to be dependent on the induction and stabilisation of LTP (for review see Lynch, 2002). So it could be hypothesised that antagonising the α7 nAChR at reinstatement

interferes with the machinery implicated in plasticity events needed to recall and reinstate morphine-CPP but not the different machinery that is needed for expression and acquisition of morphine-CPP. As extinction has been shown to cause LTD, it could be hypothesised that LTP is needed to reinstate morphine-CPP and it is this process that MLA administration disrupts.

Another consideration is the firing of the septal cholinergic input, as increases in ACh have been reported after cue exposure (Crespo *et al.*, 2006) and a time dependent effect on LTP has been reported with nicotine (Ji *et al.*, 2001a) and endogenous ACh (McGehee, 2002a). The timing of input of activation from the septal cholinergic input to the hippocampus can induce different forms of plasticity that depend solely on the timing of the input (Yakel, 2012).

The data presented in this chapter shows that morphine reinstatement significantly increases [^{3}H]-AMPA binding in the CA1 and CA2 of the ventral hippocampus, which is significantly blocked by MLA pre-treatment. In contrast there are no changes in the dorsal hippocampus. The following chapter will aim to further explore the potential difference in function across the dorsal-ventral axis. To identify a locus of effect for the behavioural data shown in chapter 3, MLA will be infused directly into the ventral and dorsal hippocampus as well as the mPFC, 15 minutes prior to reinstatement of morphine-CPP.

CHAPTER 5 INTRACEREBRAL LOCUS OF ACTION WITH RESPECT TO MORPHINE-CPP

5.1 The role of the medial prefrontal cortex and the hippocampus in cognition.

The previous chapters showed that MLA blocks morphine primed reinstatement and this causes a significant reduction of [^{3}H]AMPA binding in CA1 and CA2 of the ventral hippocampus. The work described in this chapter aims to confirm a locus for this effect using intracranial delivery of MLA. First it needs to be understood how this relates to what is known about the brain circuitry underlying motivational learning.

The medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing.

There are two distinct circuits that can operate independently in learning and memory. The HPC proper consists of the dentate gyrus (DG) and the CA1-3 fields which receive cortical input from the entorhinal cortex (EC) through the perforant pathway (PP). Its output is generated in the CA1 and the subiculum and sent back to the EC (Amaral & Witter, 1995). The subcortical connections via the fornix, a collection of fibres, connect the HPC with the septum, nucleus accumbens and the brain stem. The basolateral complex of the amygdala (BLA) has been shown to be involved in the consolidation of emotionally salient memories (McGaugh, 2004). Lesions of the amygdala and HPC proper have been shown to abolish CPP, whilst the fornix lesions enhance it (Ferbinteanu & McDonald, 2001). Although Morris *et al* (1982) throws doubt on these claims as they have shown that hippocampal proper lesions cause profound and lasting impairment in spatial navigation seen in the Morris maze task.

The medial prefrontal cortex mediates several higher order functions including decision making and goal-oriented behaviour. In particular the prelimbic (Prl) region of the mPFC has been implicated in cognition through its strong interconnections with the hippocampus, insular cortex, nucleus accumbens, basolateral nucleus of the amygdala, the mediodorsal and reuniens nuclei of the thalamus and the ventral tegmental area of the mid-brain. Lesions of these regions have been shown to

produce deficits in the delayed response tasks (Brito & Brito, 1990; Seamans *et al.*, 1995; Dalley *et al.*, 2004) and increases conditioned freezing behaviour (Frysztak & Neafsey, 1994; Vertes, 2006) as well as spatial learning and memory formation (Lavenex *et al.*, 2006). There is evidence that the nucleus reuniens of the thalamus acts as an interface between the mPFC and the hippocampus, as well as acting as an important source of afferent limbic information to the mPFC and hippocampus (for review see Vertes, 2006). In short it could be argued that the BLA signals emotional valance, the vHPC provides contextual relevance, whereas the mPFC provides action outcome information.

Certainly lesions or inactivation of these regions cause defects in CPP. Lesions of the mPFC prevent acquisition but not reinstatement of morphine-induced CPP (Hao *et al.*, 2008). Selective inactivation of the Prl and the basal amygdala attenuated cued reinstatement of extinguished cocaine-seeking behaviour in rats (McLaughlin & See, 2003). Electrical stimulation of the pre-limbic cortex (100μA) suppressed morphine induced-CPP, revealing that blocking the connection from the hippocampus to the prelimbic cortex of the mPFC may be important in reward related learning and memory (Kargari *et al.*, 2012).

Functional differences between the ventral and dorsal sub-regions of the hippocampus.

The autoradiography data shown in the previous chapter suggests that the dorsal and the ventral hippocampus play a differential role in mediating reinstatement to morphine-CPP. There is considerable evidence in the literature that suggest that the hippocampus may not be a unitary structure and the contribution it makes to cognition seems to vary along the septal-temporal axis (Moser *et al.*, 1993; Bannerman *et al.*, 2004; Fanselow & Dong, 2010). The afferent anatomical connections to the entorhinal cortex (Amaral & Witter, 1989; Dolorfo & Amaral, 1998), their efferent connections to the cortical and subcortical areas (van Groen & Wyss, 1990), their glutamate receptor expression (as discussed in the previous chapter, Pandis *et al*, 2006) as well as the response to nicotine (Abdulla *et al.*, 1996; Singer *et al.*, 2004) are all different across the septo-temporal axis. The dorsal hippocampus is defined as the 50% of hippocampal volume starting at the septal

pole; whereas the ventral hippocampus is defined as the 50% of the hippocampal volume starting from the temporal pole (Bannerman *et al.*, 2004).

The dorsal and ventral HPC main projections are different which may explain their differential roles in cognitive functioning (see figure 5.1). The dorsal (or septal) CA1 which contains the greatest density of place cells coding special location (Jung *et al.*, 1994) sends excitatory, multisynaptic, projections to the dorsal parts of the subiculum, presubiculum, and postsubiculum (Witter & Groenewegen, 1984). These areas are thought to contain cells signalling control on head positioning (Taube, 2007). Another vast projection from the dorsal HPC projects, via the postcommissural fornix, to the medial and lateral mammillary nuclei and the anterior thalamic complex, two areas heavily implicated in navigation (Taube, 2007). The dorsal hippocampus is also thought to be important for cognitive processing of visuospatial information as well as memory processing (Han *et al.*, 2003; Frankland *et al.*, 2004; Lavenex *et al.*, 2006) and certainly its most prominent projection from the CA1 is to the retrosplenial (RSP) and anterior cingulate cortices in the rat (Vogt & Miller, 1983; Van Groen & Wyss, 2003; Cenquizca & Swanson, 2007) and the monkey (Kobayashi & Amaral, 2007). This dorsal hippocampal – subiculum complex forms a cortical network with the retrosplenial and anterior cingulate cortical areas that together mediate behaviours such as learning, memory and spatial navigation.

The main difference from its counterpart is the vHPC's connectivity with the amygdala nuclei that receives main accessory olfactory sensory inputs (Cenquizca & Swanson, 2007). The ventral hippocampus and the amygdala nuclei also share bilateral connectivity with the infralimbic and prelimbic medial prefrontal cortex (Jones & Wilson, 2005; Roberts *et al.*, 2007; Hoover & Vertes, 2007). Together these regions form a series of projections, indirectly and directly, through the lateral septum, the medial and central amygdala nuclei, and bed nuclei of the stria terminalis (BNST), to innervate the areas of the hypothalamus, and are involved in endocrine control and modulating responses to key motivational behaviours such as ingestion, reproduction and defence (Dong *et al.*, 2001).

The ventral CA1, via the ventral subiculum and medial band of the lateral and medial entorhinal cortical areas, projects to the caudo-medial shell nucleus accumbens (but not the rostal parts) (Groenewegen *et al.*, 1996; Naber & Witter, 1998). The nucleus accumbens is a point of convergence for excitatory afferents arising from ventral hippocampus but also cortical and limbic regions including the basolateral amygdala, and the mPFC.

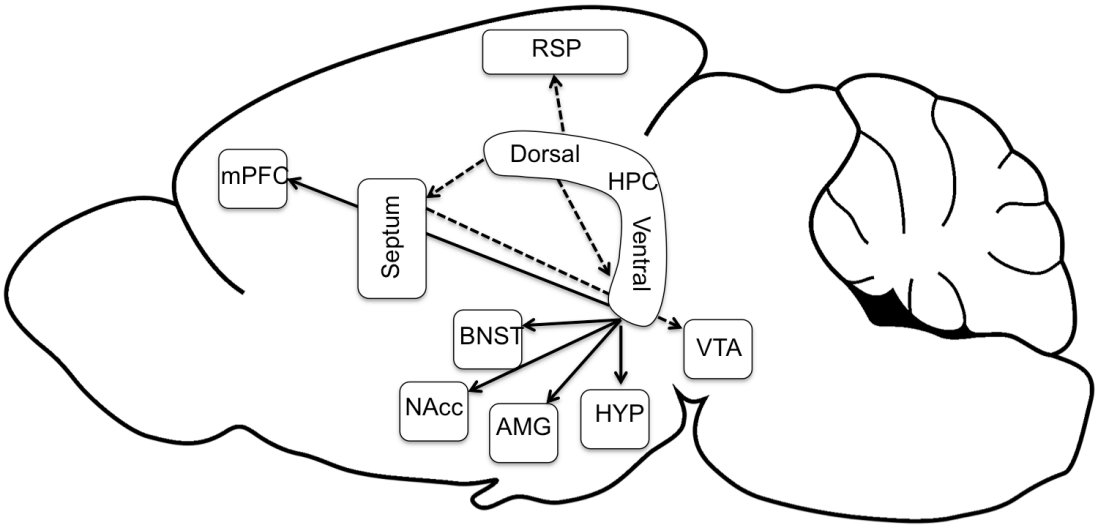


Figure 5-1 The main projections along the dorsal-ventral axis of the hippocampus.
The dorsal hippocampus projects to the retrosplenial area (RSP) of the anterior cingulate cortex then to the ventral tegmental area (VTA) via the septum. Dorsal hippocampus also sends projections to the ventral hippocampus (solid line). Projections from the ventral hippocampus include (broken line): the medial prefrontal cortex (mPFC), hypothalamus, amygdala, bed nucleus of the stria terminalis (BNST), and the VTA via the nucleus accumbens (nACC), implicating it in mediating reward learning (Tannenholz *et al.*, 2014).

Furthermore research is suggesting that the functional connectivity of the hippocampus is altered by plastic changes such as exposure to drugs of abuse, thereby shifting the efferent output of the hippocampus from dHPC (cortical) toward vHPC (limbic) influenced circuits. Stressors can also induce differential effects across the septotemporal axis of the hippocampus. For example Keralapurath *et al* (2014) found that mild stressors of their protocol alone caused metaplastic changes in the ventral hippocampus (increased LTP) and dorsal hippocampus (reduced LTP). Cocaine on the other hand only induced plasticity in the ventral hippocampus which was blocked by eticlopride (a D_2-like receptor antagonist).

Many studies have implicated the ventral but not the dorsal in controlling drug seeking behaviour to cocaine (Rogers & See, 2007). Bilateral microinjections of GABA receptor agonist (Baclofen/muscimol -0.1/1.0mM) into the ventral hippocampus attenuates cue induced as well as cocaine induced reinstatement. Conversely others have shown the importance of the dorsal hippocampus, (Rezayof *et al.*, 2006). Glutamate receptors in the dHPC have been shown to mediate the acquisition but not the expression of conditioned aversion by morphine withdrawal (Hou *et al.*, 2009). For example D-AP5 and NBQX impaired the acquisition of CPA in acute morphine dependent rats but no the expression when administered into the DHPC (Hou *et al.*, 2009). Furthermore chronic nicotine increases the number of nicotinic receptors in the dorsal hippocampus but not in the ventral hippocampus (Abdulla *et al.*, 1996).

Expression of α7 in the rodent brain

To understand the involvement of these brain regions in the MLA response, the nAChR expression must be considered. Using [^{125}I]α-BGT binding within the rodent brain expression of the α7 receptor has been shown to be high in the cerebral cortex, hypothalamus, hippocampus, inferior colliculus, and in certain brain stem nuclei (Clarke *et al.*, 1985; Fuchs, 1989; Séguéla *et al.*, 1993). The thalamus and striatum seem completely devoid of binding (Clarke *et al.*, 1985; Sargent, 1993) and there is some discrepancy over the binding in the VTA. Despite the debate regarding the selectivity of this ligand (Mogg *et al.*, 2002), there is good agreement with the distribution of α7 transcript riboprobes (Séguéla *et al.*, 1993; Dominguez del Toro *et al.*, 1994). As much evidence has accumulated for the role for the hippocampus and the mPFC (Rezayof *et al.*, 2006; Hao *et al.*, 2008; Van den Oever *et al.*, 2010; Kargari *et al.*, 2012) in cue induced reinstatement, therefore these regions will be consider in more detail.

Antatomical expression of nAChRs in the medial PFC

As in other areas of the cortex nAChRs are expressed in a layer specific manner (layers I-V)(Poorthuis *et al.*, 2012). Interneurons in all layers express nAChR but their distribution and type are different depending what layer they are expressed in (see figure 5.2). It is thought that the mPFC layer V pyramidal neurons are prominently modulated by M1 receptors (Gulledge *et al.*, 2007) whereas layer II-III pyramidal neurons are modulate primarily by α4β2 and α7. α7 nAChRs are found on pyramidal cells in layer V, and on interneurons in layer I, II/III, and V (Bloem *et al.*, 2014). The thalamic input to layer V is heavily modulated by β_2 nAChRs.

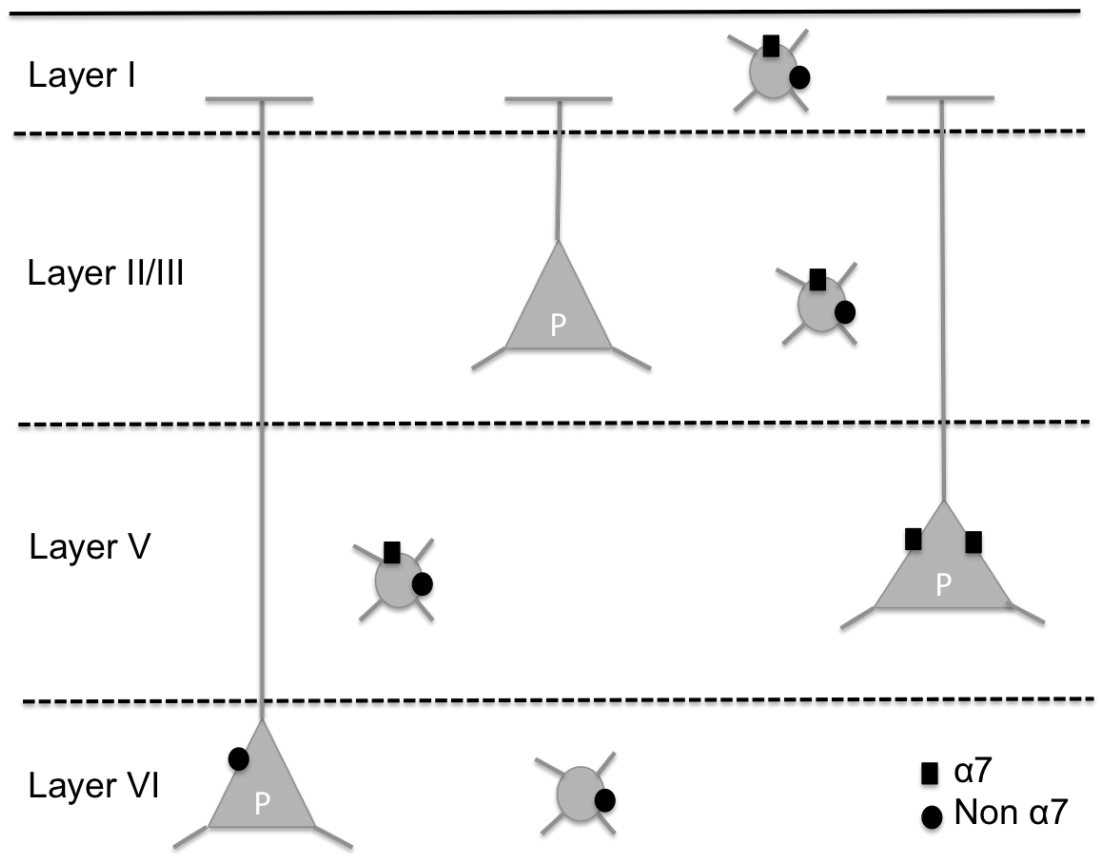


Figure 5-2 The localisation of α7 and non α7 nAChRs in a layer specific manner in the mPFC. (Bloem *et al.*, 2014).

Anatomical expression of nAChRs in the hippocampus

In situ hybridisation has shown high α7 expression in the hippocampus (Martin & Aceto, 1981; Séguéla *et al.*, 1993), although there are no reports of differences in expression between the ventral and dorsal hippocampus. Using both anti-α7 nAChR immunolabelling and α-bungratoxin binding, Fabian-Fine *et al* (2001) find α7 receptors present at nearly all synapses in the CA1 stratum radiatum pre- and post-synaptically. Low levels of α7mRNA have been found in pyramidal cells by RT PCR (Sudweeks & Yakel, 2000), however no direct nAChR-mediated excitation has been found (Frazier *et al.*, 1998).

However α7 nAChRs are thought to be present presynaptically on excitatory glutamatergic nerve terminals (Sudweeks & Yakel, 2000). The CA3 mossy fibres expressing α7, synapse with pyramidal neurons that give rise to the Schaffer collateral pathway. α7nAChR are also present on GABAergic interneurons in the hippocampus and they actually express much high levels of nAChRs than pyramidal cells (Frazier *et al.*, 1998; McQuiston & Madison, 1999; Ji & Dani, 2000; Yakel & Shao, 2004). In the septohippocampal formation α7 nAChRs are predominately expressed by neurons well positioned to modulate hippocampal theta oscillation, such as GABAergic interneurons in the hippocampus, and by both GABAergic and cholinergic septal neurons (Siok *et al.*, 2006). The anatomical expression of non α7 and α7 nAChRs are summarised in figure 5.3

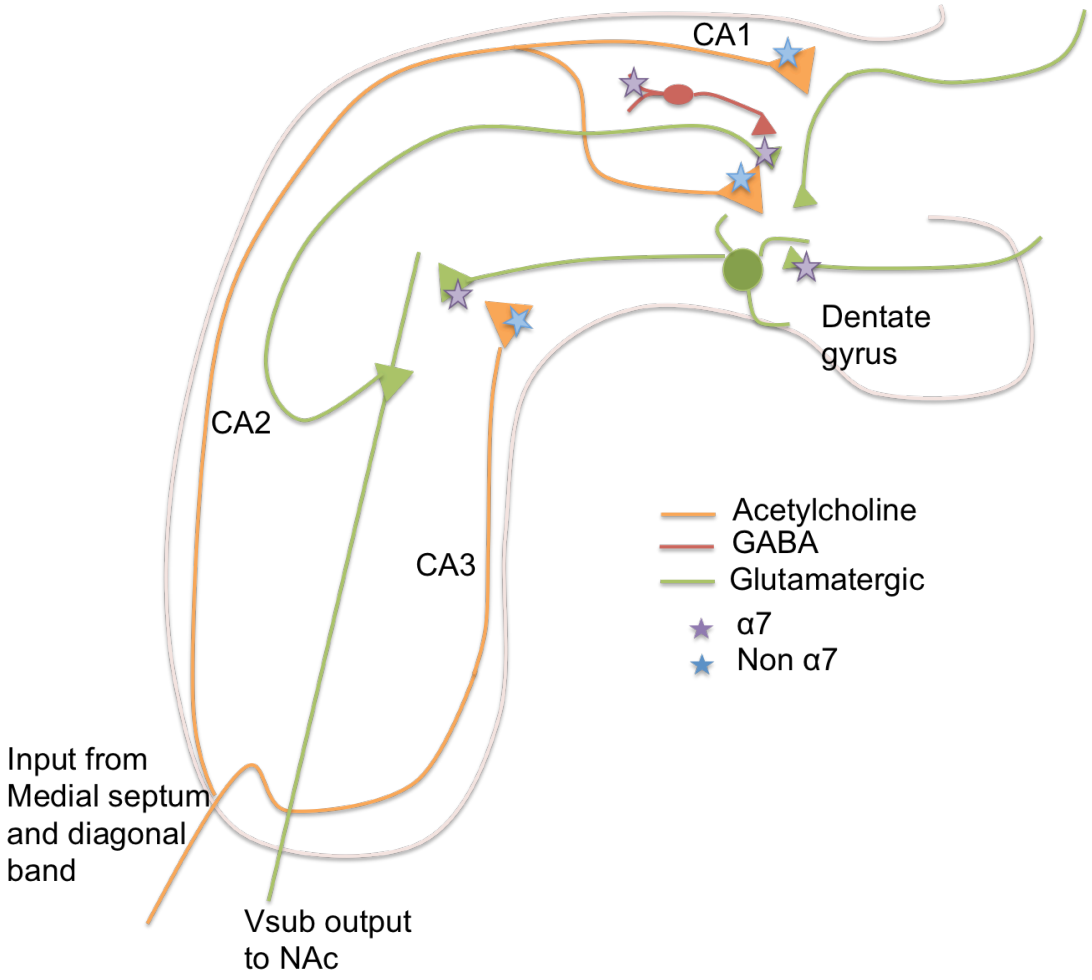


Figure 5-3 The expression of nAChRs in the ventral hippocampus.
The main cholinergic innervation from the medial septum expressing non-α7 nAChRs synapses with pyramidal neurons that express α7 nAChRs throught the CA3-1. The firing rate of these cells can be modulated by GABAergic interneurons expressing α7 nAChRs (Frazier *et al.*, 1998; McQuiston & Madison, 1999; Ji & Dani, 2000; Yakel & Shao, 2004).

Behavioural effects of infusions of nicotinic cholinergic drugs

As discussed in Chapter 1 (section 1.3) nicotine can have very diverse effects on different types of learning and memory (Kenney & Gould, 2008) and this variability maybe due to the effect on different brain regions. For example Hahn *et al* (2003) found that the effects of systemic nicotine administration could be mimicked with local bilateral infusions of nicotine into the mPFC on attention in the 5-CSRTT. The same study found no effect of nicotine infusion into the dorsal hippocampus. In an additional study they also showed that nicotine's effect on the 5-choice serial reaction time task (5-CSRTT) was dependent on α7 rather than β2* nAChRs using co-application of DHβE, a β2 containing-nAChR antagonist, and MLA (Hahn *et al.*,

2011) but conversely nicotine attentional effects were found to be mediated with specific β2 agonists but not α7 agonists (Young & Geyer, 2013). Raybuck & Gould (2010) found varying effects of nicotine and antagonists on trace and contextual fear learning when infused bilateral into the hippocampus and mPFC. Nicotine infusion impaired fear learning in dHPC and vHPC but not in the mPFC it impaired trace fear learning but had no effect on contextual fear learning. They also found that MLA and DHβE also enhanced trace fear conditioning in the mPFC.

Opiate reward has been shown to be dependent on learning and memory and studies discussed previously suggest a role for α7 nAChRs in the mPFC and the hippocampus in mediating this response. Nicotinic antagonists and agonists have been shown to alter LTP and evidence suggests they may be particularly important in the formation of the association made between the reward and the environment.

5.2 Aim of Chapter

The aim of the work described here was to determine the locus of action of MLA in combatting reinstatement of morphine-CPP. To address this aim MLA was delivered intracerebrally prior to morphine-primed reinstatement. Knowledge of the circuitry underpinning motivational learning, together with data from the autoradiography study (Chapter 4) identified mPFC, dHPC, vHPC as candidate regions to examine. As intracerebral administration requires implantation of an in-dwelling cannula, it was necessary to carry out this study in rats. Therefore the initial aim was to reproduce reinstatement to morphine-CPP and its sensitivity to systemic MLA in rats.

5.3 Results

Validation of reinstatement of morphine-CPP in the rat

Morphine priming reinstates morphine-CPP in male Wistar rat

To ensure the effect of reintroducing the unconditioned stimulus after extinction are comparable across species, male Wistar rats underwent conditioning (morphine 5mg/kg, s.c), extinction and received either a saline or morphine (2.5mg/kg, s.c) priming dose immediately before the reinstatement trial (figure 5.4). A repeated measures one-way ANOVA showed that there was a non-significant effect of treatment ($F_{(1,22)}$=1.43, p=0.245) but a significant effect of test ($F_{(3,66)}$=4.02, p=0.011) showing significant acquisition of morphine CPP in both groups. *Post-hoc* comparisons revealed significant difference between reinstatement in the two treatments (morphine 2.5mg/kg: 267.0±81.2s in DP side, saline 1ml/kg: -55.9 ±82.2s in DP side, p=0.003, n=8/treatment group). Only the morphine treated group reinstated (p=0.007 vs p=0.34). An unpaired t-test revealed no significant difference in the distance moved during reinstatement trial, p=0.2241, n=26/treatment group).

MLA inhibits drug-primed reinstatement to morphine-CPP in male Wistar rats

To test whether MLA has an effect on the reinstatement of morphine-CPP by morphine drug priming in male Wistar rats, MLA (4mg/kg, s.c) was administered 20 minutes prior to the morphine-priming dose. Figure 5.5 shows that the effect of MLA on the reinstatement of morphine-CPP. A repeated one-way ANOVA revealed no significance in the effect of treatment ($F_{(1,49)}$ = 0.70, p=0.408) but a significant effect of test ($F_{(4,176)}$ = 3.96, p=0.004, n=26/treatment group). *Post-hoc* pairwise comparisons revealed that only animals pre-treated with saline significantly reinstated (saline: 25±23s in DP paired side at extinction vs 112.0±46s at reinstatement (p=0.007); MLA: 18±23s in DP paired side at extinction vs 35±46s at reinstatement, p=0.667, n=26/treatment group). The time spent in drug paired side was significantly different between the two treatments (p=0.024). An unpaired t-test revealed no significant difference in the distance moved during reinstatement trial, p=0.0965, n=26/treatment group).

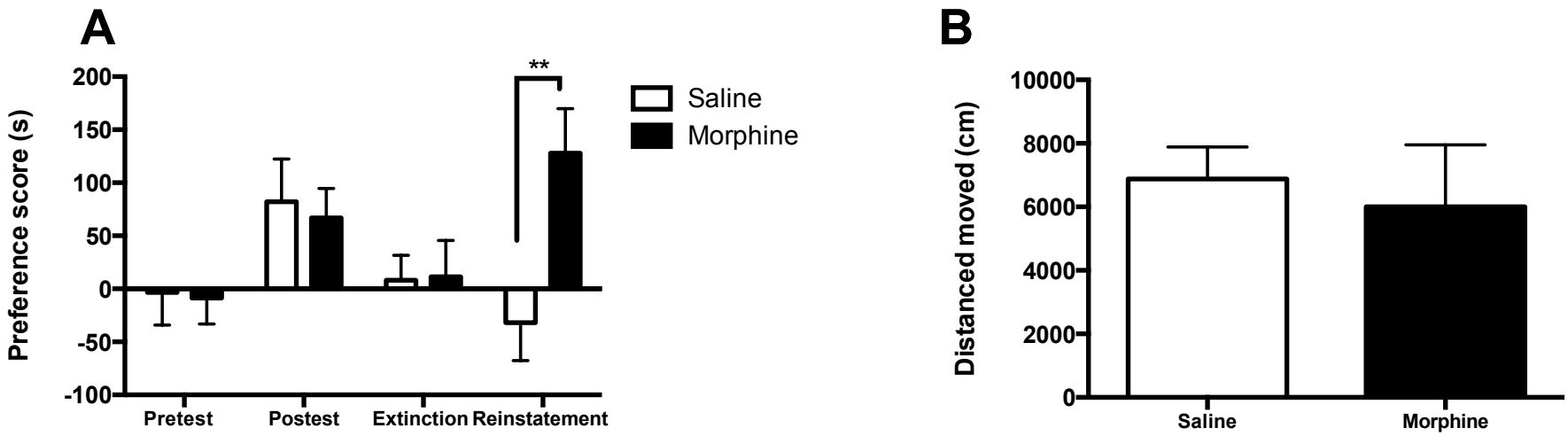


Figure 5-4 A) Morphine priming reinstates morphine-induced CPP in Wistar rats.
Rats underwent acquisition and extinction of morphine-CPP, before reinstatement, where rats were either primed with morphine (2.5mg,kg,s.c) or saline (10ml/kg, s.c). All data shown as mean±SEM. There was only significant reinstatement in rats primed with morphine (p=0.007 vs p=0.34). B) An unpaired t-test revealed no significant difference in the distance moved during reinstatement trial, p=0.2241, n=26/treatment group).

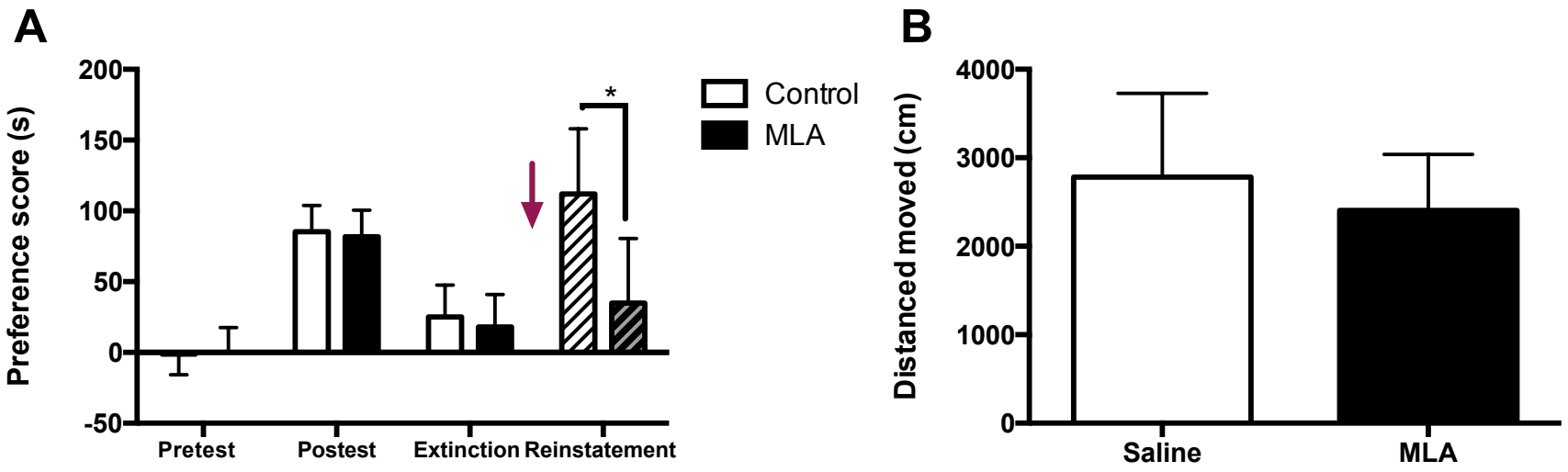


Figure 5-5 MLA inhibits drug primed reinstatement of morphine CPP in Wistar rats.
Rats underwent acquisition and extinction of morphine-CPP, before MLA reinstatement, where rats were either pre-treated with MLA (4mg/kg, s.c.) or saline (10ml/kg, s.c.) before morphine priming (2.5mg,kg,s.c). All data shown as mean±SEM. Only animals pre-treated with saline significantly reinstated (saline: 25±23s in DP paired side at extinction vs 112.0±46s at reinstatement (p=0.007); MLA: 18±23s in DP paired side at extinction vs 35±46s at reinstatement, p=0.667, n=26/treatment group). The time spent in drug paired side was significantly different between the two treatments (p=0.024). B) There was no difference in the distance moved between the two treatment groups (p=0.0965, n=26/treatment group).

The effect of intracranial infusion of MLA into the mPFC, DHPC and VHPC on drug primed reinstatement to morphine-CPP

Pilot study

A pilot study was conducted to confirm the placement of the cannula and to test whether the surgery protocol interfered with the conditioned place preference protocol (figure 5.6). 12 animals in total were trained in CPP. All animals acquired CPP and all reach extinction. Animals were then randomly allocated treatment groups: half the group undergoing cannulation surgery (2 had mPFC, 2 dHPC and 2 vHPC bilateral cannula implanted), whilst 6 had a rest week at the end of which they were singly housed. One week post surgery animals underwent either saline infusion of s.c saline administration 15 minutes prior to morphine (2.5mg/kg, s.c.) primed reinstatement.

A repeated measures ANOVA revealed no significance in the effect of treatment ($F_{(1,5)}$ = 3.63, p=0.115) but a significant effect of test ($F_{(3,24)}$ = 10.763, p=<0.001, n=6/treatment group). Post-hoc pairwise comparisons revealed no difference in time spent in drug paired side for the surgery vs control animals (surgery: -4.8±89s in DP paired side at extinction vs 318.0±54.8s at reinstatement; controls: -0.3±112.2s in DP paired side at extinction vs 424.6±87.1s at reinstatement (p=0.195).

An unpaired t-test revealed no significant difference in the distance moved during reinstatement trial, p=0.0965, n=26/treatment group).

The brains were sectioned after an infusion of dye to confirm placement of the cannula, revealing 2 rats with bilateral placements in the mPFC, 2 with bilateral placements in the ventral hippocampus, and only 1 of the 2 rats had bilateral placements in the dorsal hippocampus (figure 5.7).

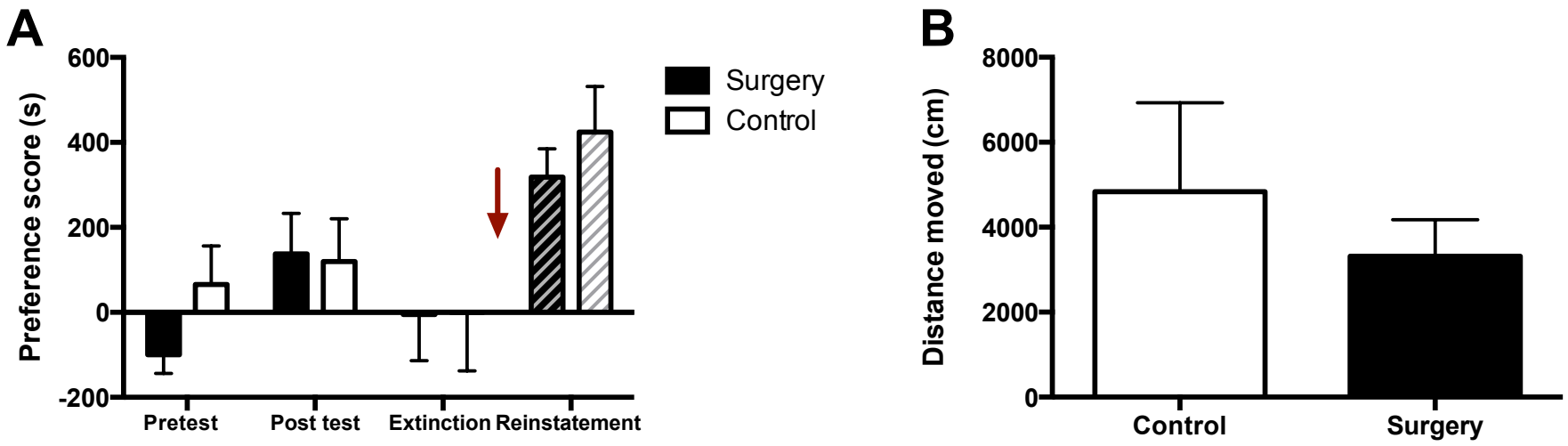


Figure 5-6 Surgery to implant indwelling cannula had no effect on reinstatement to morphine CPP in Wistar rats.
After the acquisition and extinction of morphine-CPP, 6 Wistar rats under surgery for the implanatation of bilateral cannula, whilst 6 remained in their home cages. All data shown as mean±SEM. One week post surgery cannulated animals were infused with saline 20mins prior to their morphine reinstatement dose (2.5mg/kg, s.c), whilst control animals received saline (10ml/kg, s.c) **A)** There was no difference in time spent in drug paired side for the surgery vs control animals (saline: 25±23s in DP paired side at extinction vs 112.0±46s at reinstatement (p=0.195, n=6/treatment group). **B)** Surgery had no effect on the distance move during the reinstatement trial.

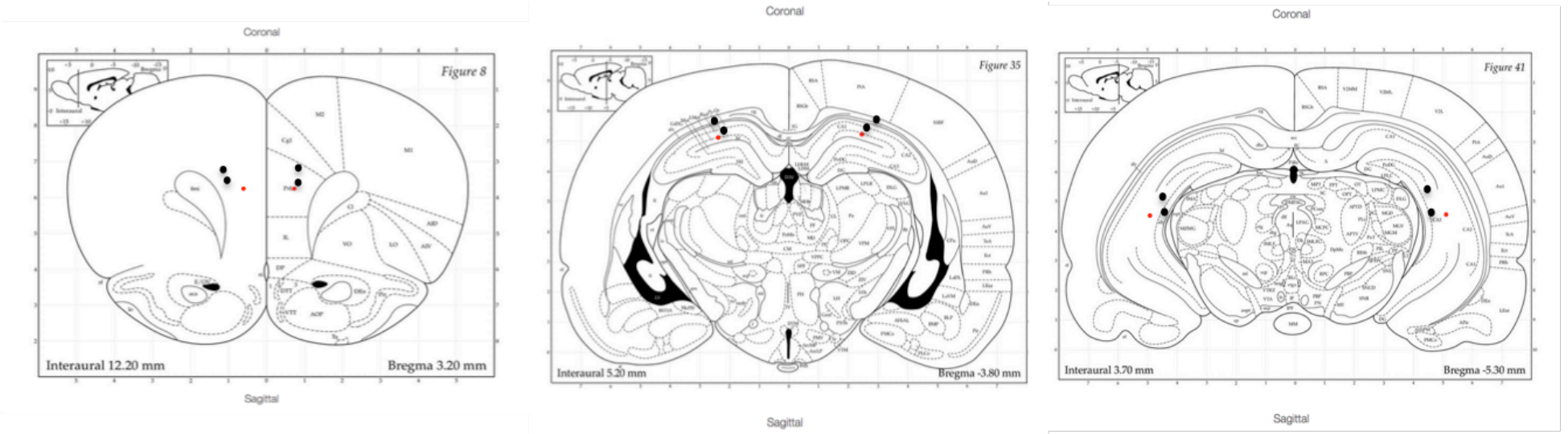


Figure 5-7 Schematic showing the placements of the cannula tips determined by dye infusions.
Dye infusions of 0.5 µl of brilliant blue revealed 2 rats with bilateral placements in the mPFC, 2 with bilateral placements in the ventral hippocampus, but only 1 of the 2 rats had bilateral placements in the dorsal hippocampus. Small dot represents target coordinate. All placements determined using a rat brain atlas (Paxinos and Watson, 2007).

MLA infusion

Animals underwent acquisition and extinction of morphine-CPP, before surgical implantation of cannula. A week post surgery and daily habituation to the infusion procedure animals were infused with either saline or MLA (6.75ug/hemisphere, at a rate of 0.6ul/min for 4 minutes with a 4 minute wait before cannula removal). After 15 minutes in home cage in the experimental room animals were given morphine (2.5mg/kg, s.c) and placed in the CPP boxes for the reinstatement trial. Time spent in the DP side and their locomotion was recorded. After the reinstatement trial animal were sacrificed and cannula placements were verified by an infusion of 0.5 ul of brilliant blue dye.

A total of 52 rats were used in this study. Of these 16 rats had bilateral placements within the dorsal hippocampus, 16 had bilateral placements in the ventral hippocampus and 16 had bilateral placements in the medial prefrontal cortex, 3 rats were lost through sickness. The tips of the cannula were located for bilateral infusion flow into the dHPC, vHPC and mPFC in all animals, but some dye was also found in the ventricles in the dHPC infusions. A schematic diagram illustrating the distribution of injection cannula placements in the brains of rats and photomicrographs of representative cannula tracks are included in figure 5.10 (saline treated) and figure 5.11 (MLA treated).

A one-way ANOVA with repeated measures showed a significant effect of morphine-CPP ($F_{(2,94)} = 3.38$, p=0.0381) but no significant effect of treatment group ($F_{(1,47)} = 0.03$, p=<0.8657, n=25/treatment group). Showing there was no difference in initial CPP training and extinction between animal groups that went on to receive morphine reinstatement and local MLA or saline infusions (figure 5.8). Figure 5.9A shows the effect of MLA infusion into the mPFC, DHPC, and VHPC. A multiple t-test showed no effect of MLA intra-mPFC (p=0.70, n=8) or intra-dHPC (p=0.82, n=8) on the time spent in drug paired side during reinstatement. However there was a significant effect of MLA intra-vHPC (p=0.012, n=8). This shows that MLA delivered intracranially into the ventral hippocampus, but not the dorsal hippocampus or mPFC can significantly block reinstatement to morphine-CPP. There was no

effect of intracranial MLA on locomotion seen in any of the sites (figure 5.9B mPFC: p=0.064; dHPC: p=0.24; vHPC: p=0.29).

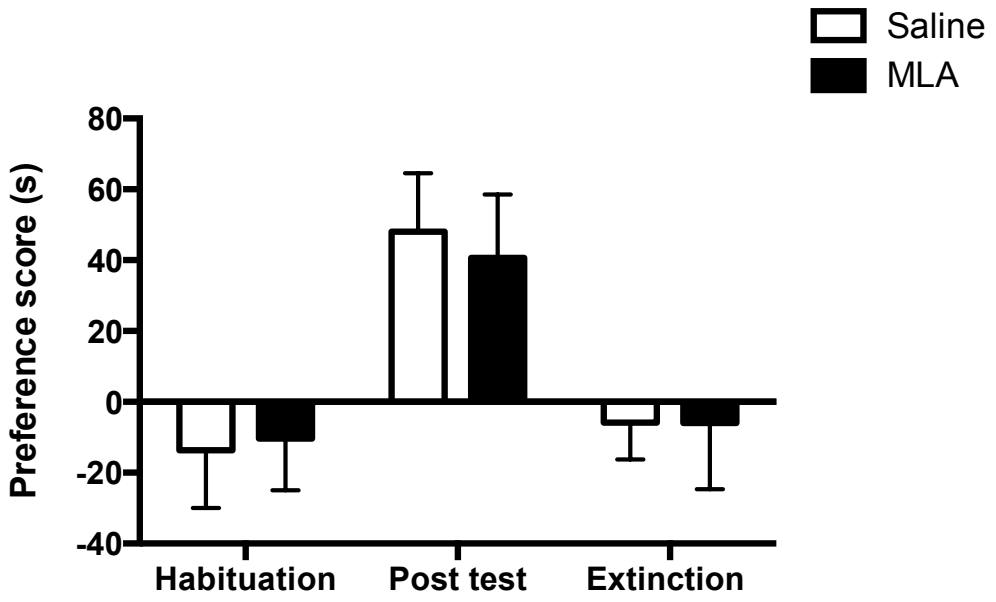


Figure 5-8 Prior to surgery all rats were conditioned to morphine and successfully extinguished with repeated saline pairings.
A one-way ANOVA with repeated measures showed a significant effect of morphine-CPP ($F_{(2,94)}$ = 3.38, p=0.0381) but no significant effect of treatment group ($F_{(1,47)}$ = 0.03, p=<0.8657, n=25/treatment group). All data shown as mean±SEM. Showing there was no difference in initial CPP training and extinction between animal groups that went on to receive morphine reinstatement and local MLA or saline infusions.

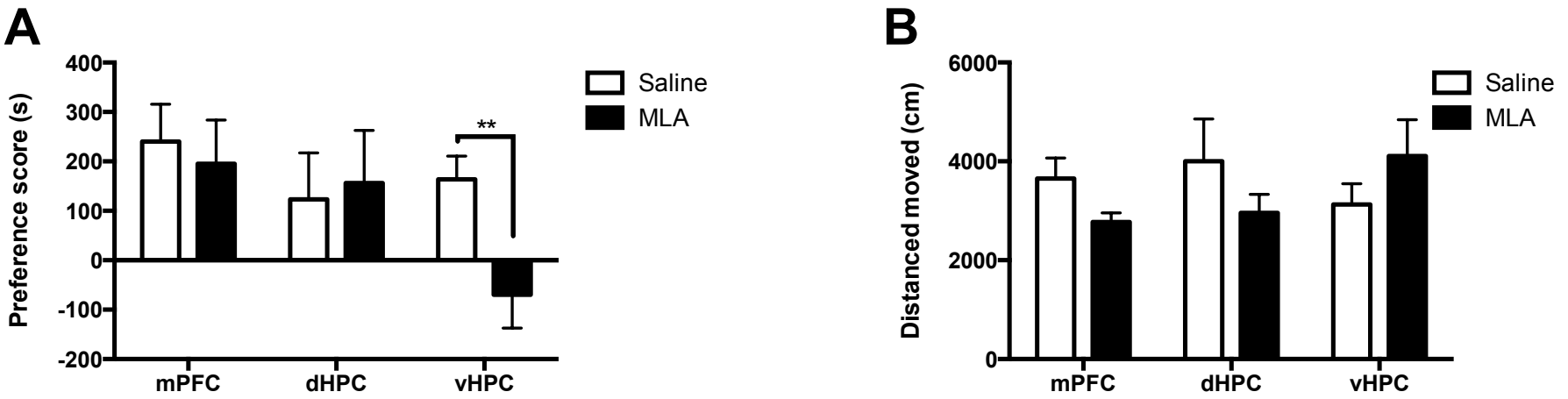


Figure 5-9 Intra-vHPC MLA infusion significantly reduces reinstatement to morphine-primed morphine-CPP.
Rats were conditioned and extinguished as previously reported, after surgical implantation of bilateral cannula animals were allowed 1 week for recovery, during which the animals underwent daily habituation to the infusion procedure. MLA or saline at the same volume was dosed at 6.74μg/hemisphere into the mPFC, dHPC, and vHPC, 20 minutes prior to a morphine reinstatement dose, time spent in drug paired side was recorded. All data shown as mean±SEM. A) A multiple t-test showed no effect of MLA intra-mPFC (p=0.70, n=8) or intra-dHPC (p=0.82, n=8) on the time spent in drug paired side during reinstatement. However there was a significant effect of MLA intra-vHPC (**p=0.012, n=8). B) There was no effect of intracranial MLA on locomotion seen in any of the sites (mPFC: p=0.064; dHPC: p=0.24; vHPC: p=0.29).

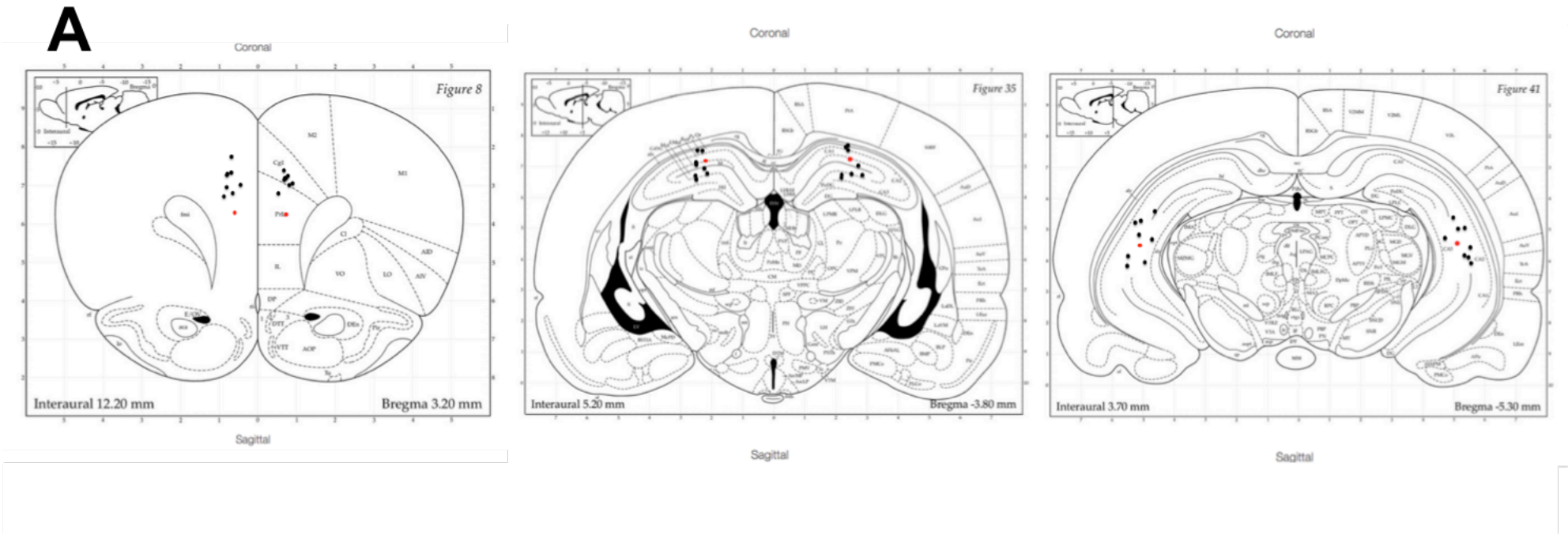


Figure 5-10 A schematic diagram illustrating the distribution of injection cannula placements in the brains of saline treated rats.
Dye infusions of 0.5 µl of brilliant blue reveal all cannula placements were within the mPFC, dorsal HPC and ventral HPC. Small dot represents target coordinate. All placements determined using a rat brain atlas (Paxinos and Watson, 2007).

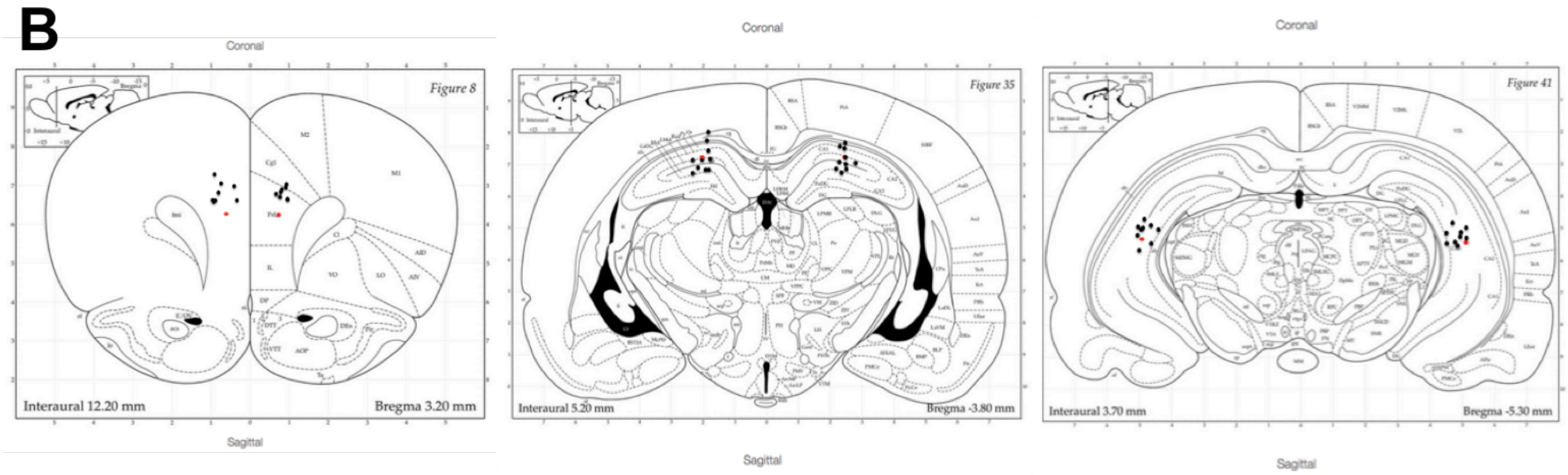


Figure 5-11 A schematic diagram illustrating the distribution of injection cannula placements in the brains of MLA treated rats.
Dye infusions of 0.5 µl of brilliant blue reveal all cannula placements were within the mPFC, dorsal HPC and ventral HPC. Small dot represents target coordinate. All placements determined using a rat brain atlas (Paxinos and Watson, 2007).

5.4 Discussion

MLA causes a partial reduction in reinstatement in Wistar rats

Importantly the data presented in this chapter extends the finding that MLA, an α7 nAChR antagonist, specifically reduces reinstatement to morphine-CPP in C57BL/6J mice (see chapter 3) as the results suggests the same effect in Wistar rats. This is the first experiment of its kind and it is interesting that this effect is very similar in both species.

Intracranial delivery of MLA – Experimental considerations

A primarily consideration for the validity of this study is the dose used. Concentrations in the mM range are frequently used in intra cranial administration (Miczek *et al.*, 1985) to account for the losses of the compound within the tissue. MLA has a large molecular weight (874g/mol) and is not particularly lipophilic due to its norditerpenoid rings and charge and consequently it can stick to tissue. Furthermore the dose used in this chapter was previously used successfully in the rat (Levin, 2002; Addy *et al.*, 2003) and the mouse (Raybuck & Gould, 2010). Another consideration is the effect of non-specific effects of MLA at high doses as there is evidence that it may act at α3 and α6 subunits (Mogg *et al.*, 2002). However these subunits are often co-localised and mainly expressed in catecholaminergic areas, such as the VTA, substantia nigra and medial habenula and are not highly expressed in the mPFC or hippocampus. Furthermore there was no effect on locomotion or any evidence of non-selective effects that have been previously described (Chilton *et al.*, 2004; Tinsley *et al.*, 2011). It is important to account the possibility that the infused drugs may have spread to other areas of the brain. All cannula placements were located in the target regions and were verified by an infusion of 0.5ul of brilliant blue dye. The tips of the cannula were located for bilateral infusion flow into the required brain region in all animals, but some dye was also found in the ventricles in the DHPC infusions. Tissue damage from the cannula is another important consideration as lesions of all three of these regions has been shown to attenuate reinstatement to drug seeking (Ferbinteanu & McDonald, 2001; Hao *et al.*, 2008). However, in the pilot study we showed that stereotaxic placement of bilateral cannula into the mPFC,

dHPC and vHPC had no effect on the reinstatement of CPP. Therefore the cannula placement was not impairing the functioning of these regions in morphine CPP.

MLA infusion into the ventral but not the dorsal or the mPFC significantly inhibited reinstatement to morphine CPP.

A functional dissociation of the dorsal and ventral hippocampus?

The functional dissociation between the dorsal and ventral hippocampus we see here has been previously observed in fear conditioning (McEown & Treit, 2010) in cocaine place preference (Meyers *et al.*, 2003), and spatial learning (Pothuizen *et al.*, 2004). Relative to its dorsal counter part the ventral hippocampus has greater output connections with the prefrontal cortex, bed nucleus of the stria terminalis, and the amygdala (Henke, 1990; Ishikawa & Nakamura, 2006; Hoover & Vertes, 2007) as well as the nucleus accumbens (Groenewegen *et al.*, 1996; Naber & Witter, 1998). These connections with these areas thought to be critically involved in reward processing, may explain our result. The binding data presented in Chapter 4 of this thesis also provides support for this dissociation between the dHPC and vHPC as both NMDA and AMPA binding levels are higher in the dorsal hippocampus than the ventral hippocampus, and is confirmed elsewhere (Pandis *et al.*, 2006).

Can α7 nAChRs modulate synaptic plasticity in the hippocampus?

It is interesting to note that activation of nicotinic receptors in the hippocampus can facilitate the induction of LTP and antagonists can inhibit it. Taken together with the results showing that MLA blocks the morphine reinstatement increase in [^{3}H]AMPA binding (Chapter 4) it therefore seems a likely hypothesis that intra-vHPC injections of nAChR antagonists inhibit learning and memory, which prevents the recall of an association made between a drug and a context. Extinction has been shown to interrupt LTP and furthermore LTP is required for reinstatement (Portugal *et al*, 2014). It has been reported that hippocampal ACh levels in rats and mice increase above baseline immediately after lever pressing for food reward (Orsetti *et al.*, 1996) and assuming this is true for other paired contexts this action of this ACh may be needed for the reinstatement of CPP. Considerable evidence supports the notion that the hippocampus may be required for the integration of the representation of

environmental stimuli and internal responses elicited by a reinforcer experienced in that specific environment (White & McDonald, 2002). Thus, the present results may support the suggestion that blocking α7 nicotinic receptors could interfere with contextual learning of CPP. Together, this indicates that the injections of cholinergic agents into the ventral hippocampal region could interfere with both the rewarding properties of morphine, as well as with the mnemonic processes underlying CPP.

5.5 Conclusion

As learning and memory plays an important role in the development of opiate reward (White, 1996; Lu *et al.*, 2002). It seems reasonable to hypothesise, that in the experiments conducted in this thesis, MLA when administered either systemically or intracranially into the vHPC can inhibit processes involved in learning and memory critical for reinstatement of morphine CPP. In particular the hippocampus has been shown to be involved in integrating representations of environmental stimuli and internal response elicited by reinforcers experienced in that specific environment.

CHAPTER 6 GENERAL DISCUSSION

The work described in this thesis has shown that antagonism of the α7 nAChR selectively inhibits reinstatement but has no effect on the reconsolidation, expression and acquisition of morphine-CPP. Reinstatement of morphine-CPP induced increased [^{3}H]AMPA binding in the ventral hippocampus, and these increases were significantly reduced by pre-treatment with MLA before the reinstatement trial. Finally infusion of MLA into the ventral hippocampus, but not the dorsal hippocampus or medial prefrontal cortex, inhibited reinstatement of morphine-CPP. This discussion will hypothesise how this effect is mediated and speculate upon as of yet unanswered questions and how they may be addressed in future experiments.

6.1 Putative model for the drug primed reinstatement of morphine CPP

During acquisition of conditioned place preference (CPP) morphine acts on μ-opioid receptors on GABAergic neurons in the ventral tegmental area (VTA) inhibiting GABA release. This lifts the GABAergic inhibition of dopamine cells (Johnson & North, 1992), resulting in an increase of DA release in the nucleus accumbens which mediates the reinforcing nature of the drug. Repeated contextual administration of the drug leads to long-lasting changes within the reward circuit including changes in excitatory neurotransmission (Thomas & Malenka, 2003). Glutamatergic (Farahmandfar *et al.*, 2011a), acetylcholinergic (Neugebauer *et al.*, 2013) and GABAergic tone (Jolas *et al.*, 2000) change inducing long term potentiation, increases in AMPA glutamate receptor responsiveness. During extinction training further changes occur, such as disruption to the LTP (Portugal *et al.*, 2014) and formation of an inhibitory memory. Consequently the drug-associated memory is altered and a new memory is formed supressing the conditioned response (spending more time in the drug paired side). During reinstatement exposure to a priming dose of the drug causes increased glutamatergic and dopaminergic signalling leading to plasticity changes that restore LTP (Morón *et al.*, 2010; Portugal *et al.*, 2014) and the memory for the drug associated cues is recalled, consequently triggering reinstatement (see summary figure 6.1).

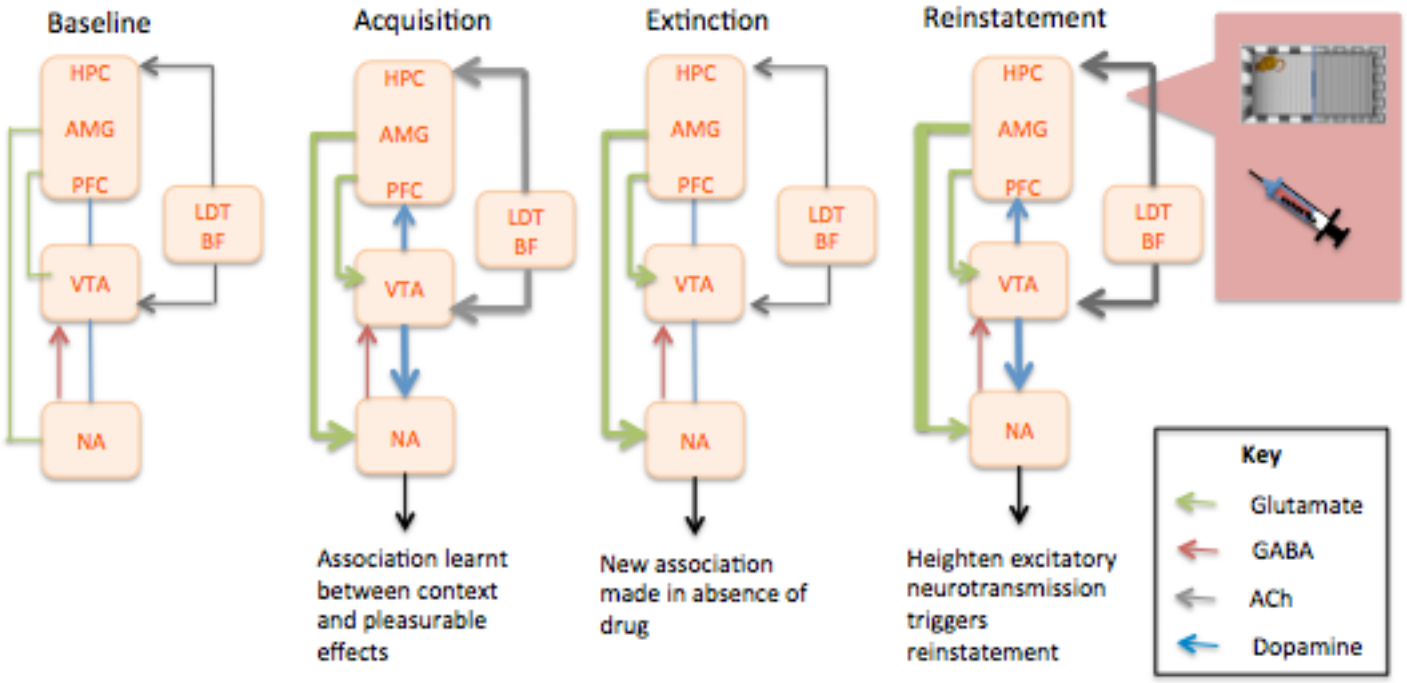


Figure 6-1 Putative model for the drug primed reinstatement of morphine-CPP.
At baseline, acetylcholine and dopamine undergo tonic firing maintaining basal tone. During administration of morphine, DA cells switch to burst firing leading to DA accumulation in the NAc which mediates the pleasurable effects of the drug. Repeated drug administration leads to plastic changes in the reward circuit mediated through changes in glutamate receptors. During extinction training further changes occur, such as disruption to the LTP. Consequently the drug-associated memory is disrupted, and the conditioned response is not expressed (spending more time in the drug paired side). During reinstatement exposure to a priming dose of the drug causes increased glutamatergic and dopaminergic signalling leading to plasticity changes that restore LTP and the memory for the drug associated cues and consequently triggering reinstatement

6.2 Putative model for the involvement of α7 nAChRs in reinstatement to morphine CPP

Due to the connectivity of the ventral hippocampus with other areas of the reward system and its cholinergic innervation from the medial septum, it could be hypothesised that this area is in a prime position to influence plastic changes occurring in response to morphine-CPP (Christie *et al.*, 1987; Totterdell & Smith, 1989; Burton *et al.*, 2009). In particular it is thought that hippocampal theta oscillations are particularly important in learning significance of drug paired contextual stimuli (Wallenstein & Hasselmo, 1997; Bland & Oddie, 2001; Luo *et al.*, 2011), and α7 nAChRs can alter these oscillations (Siok *et al.*, 2006; Tang *et al.*, 2011a; Yakel, 2012). α7 receptors are present on mossy fibre terminals that make direct contact with pyramidal neurons that give rise to the Schaffer collateral pathway, therefore directly influencing excitatory output of this region (Fujii & Sumikawa, 2001; Ji *et al.*, 2001b; McGehee, 2002a; Cobb & Davies, 2005; Maylie & Adelman, 2010). Diffuse increases in ACh induced by priming dose of morphine and exposure to a drug-paired environment may be sufficient to shift α7 nAChRs out of their desensitised state into an activated state or vice versa depending on tone and brain region (Fiserová *et al.*, 1999; Pych *et al.*, 2005; Goldberg & Reynolds, 2011). Activation causes increases in Ca^{2+} in presynaptic terminals causing increases in glutamate release and increased frequency of postsynaptic excitability and altering synaptic plasticity which is thought to be required for reinstatement (Portugal *et al.*, 2014). Furthermore GABA-containing interneurons which express post synaptic α7 nAChRs make direct contact with pyramidal cells to inhibit their firing rate, depending on cholinergic tone nAChRs can either cause disinhibition or inhibition of these pyramidal neurons, thereby modulating theta rhythm needed for LTP. Blocking α7 nAChRs in the ventral hippocampus prevents reinstatement, by blocking increase excitatory neurotransmission and consequently altering the theta rhythm required for the formation of LTP and the expression of drug cued responses, such as spending more time in the drug-paired side (see figure 6.2 for summary diagram).

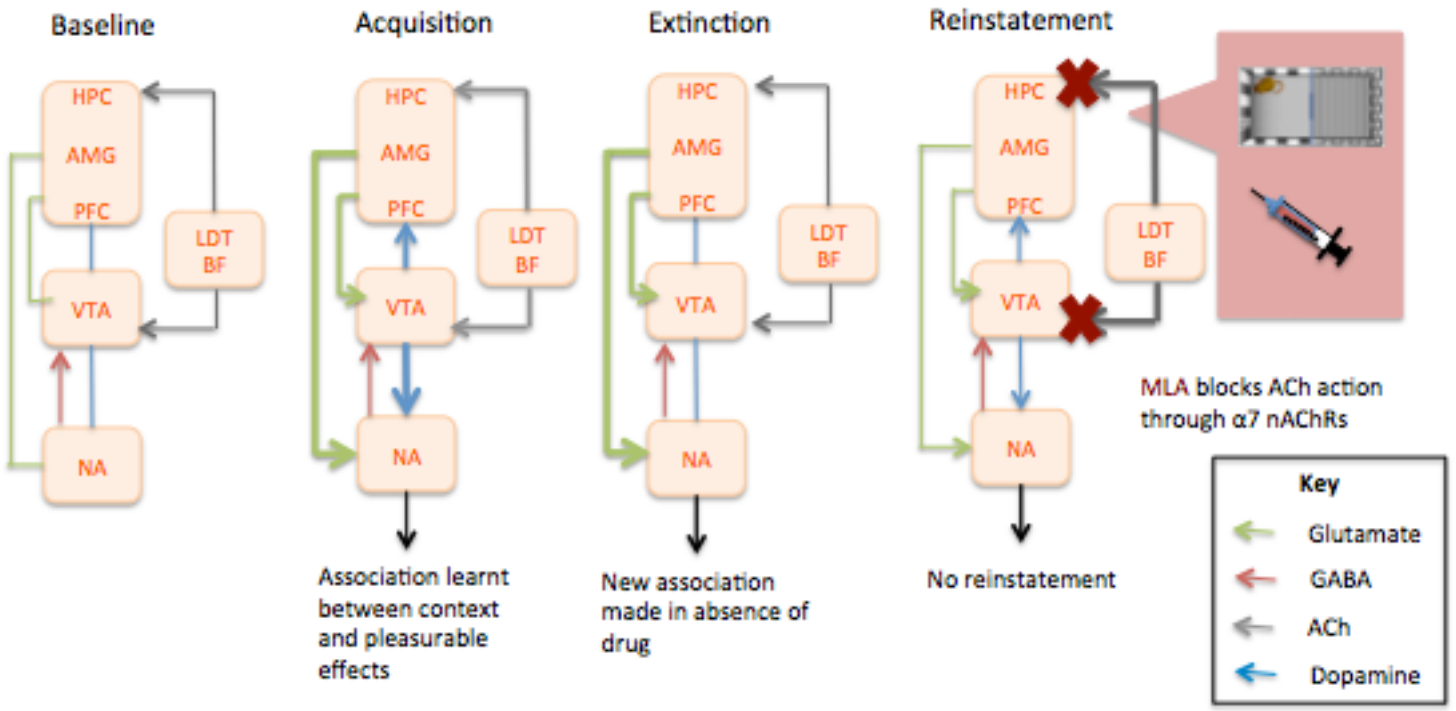


Figure 6-2 Putative model for the role of α7 nAChRs in drug primed reinstatement of morphine-CPP.
At baseline, acetylcholine and dopamine undergo tonic firing maintaining basal tone. During administration of morphine, DA cells switch to b urst firing leading to DA accumulation in the NAc which mediates the pleasurable effects of the drug. Repeated drug administration leads to plastic changes in the reward circuit mediated through changes in glutamate receptors. During extinction training further changes occur, such as disruption to the LTP. Consequently the drug-associated memory is disrupted, and the conditioned response is not expressed (spending more time in the drug paired side). During reinstatement exposure to a priming dose of the drug causes increased glutamatergic and dopaminergic signalling leading to plasticity changes that restore LTP and the memory for the drug associated cues and consequently triggering reinstatement. MLA blocks the action of ACh within the ventral hippocampus thereby blocking the excitatory and inhibitory signalling required for LTP and expression of drug cued behaviour.

6.3 Further work

Is the change in [^{3}H]AMPA binding an increase of postsynaptic protein?

The work done in this thesis shows that morphine-induced reinstatement increases [^{3}H]AMPA binding and this is significantly reduced by MLA pre-treatment in the ventral hippocampus. However, as discussed in chapter 5, it is unknown whether this is an increase in functional synaptic receptors, which is central to the hypothesis outlined above. Temporally, it is unlikely that this change in binding is a result of increase in *de novo* total receptors as the behavioural test only occurred 30 minutes prior to sacrifice, but it is feasible that it is an increase in insertion (through lateral diffusion from extrasynaptic site or vesicles) or a decrease in the removal of extant AMPA receptors. As discussed in Chapter 5 of this thesis there are further techniques that could identify where these changes in glutamate receptors are occurring. A crosslinking assay as developed by Boudreau and Wolf (2005) utilises BS^3 to selectively cross-link cell surface receptors, forming high molecular weight aggregates, whereas intracellular receptors are not modified. Thus, surface and intracellular receptor pools can be distinguished based on molecular weight using SDS-PAGE and western blotting (Boudreau & Wolf, 2005). Work done within our laboratory validated this protocol (Wright, unpublished), to confirm that the high molecular weight band is an accurate measure of surface expressed protein and that BS^3 does not access the intracellular antigen. BS^3 cross-linking measures surface and intracellular pools of GluR1. The nucleus accumbens was dissected from a naive C57BL 6J mouse using a brain matrix and a custom made puncher to cut a section with a diameter of 2mm. The punch from one hemisphere was cross-linked, whereas the other was not, generating paired samples that were then immunoblotted for GluR1, GluR2/3 and phosphoERK.

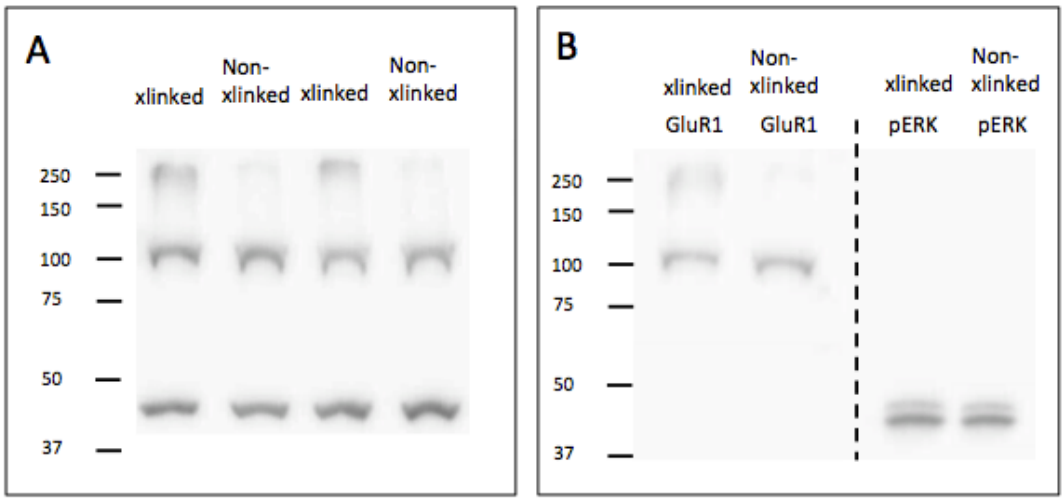


Figure 6-3 BS3 crosslinking measures surface and intracellular pools of GluR1.
A) Paired dissected NaC punches from a niave mouse, were incubated with or without the crosslinker. Samples were then blotted for GluR1. The crosslinked tissue (Xlinked) shows both a high (surface expressed) and monomeric (intracellular) molecular weight band, whereas the non crosslinked (non-xlinked) only yields a monomeric band. **B)** Only proteins that are expressed intracellularly and on the cell surface yield a high molecular weight band. Crosslinked tissue blotted for pERK revealed only a monomeric band as hypothesised.

The immunoblot shows that cross-linked tissue revealed both a high molecular weight band and a monomeric molecular weight band, whereas only the monomeric molecular weight band was detected in the non cross-linked tissue (Fig 6.3). The presence of low molecular weight monomeric bands at the expected molecular weight for phosphorylated-ERK (only expressed intracellularly) showed that BS^3 does not cross-link other intracellular proteins. This protocol could then be used to examine the changes in NMDA and AMPA subtypes, as these are known to have different roles in mediating plasticity. For example GluR2 containing AMPA receptors are known to be impermeamble to Ca^{2+}, therefore this subunit is a key determinant of AMPAR function, and these receptor undergo activity driven sub-unit switching (Billa *et al.*, 2010). Experiments using GluR1:GluR2 ratios after MLA reinstatement would give interesting insights to the behaviourally induced plasticity before and after reinstatement. Extinction of morphine dependent conditioned behaviour is associated with increase phosphorylation of the GluR1 subunit (Billa *et al.*, 2009) and withdrawal to benzodiapine has been shown to increase phosphorylation at CA1 hippocampal synapses (Das *et al.*, 2008). Furthermore the regulation of receptors occurs through phosphorylation at different sites, for example

Protein kinase A (PKA) activation strongly increases exocytosis of AMPARs by direct phosphorylation of AMPAR (Man *et al.*, 2007). Therefore phosphorylation of these proteins could also be investigated within this crosslinking paradigm.

Subcellular fractionation experiments could be also be done to elucidate where the changes in glutamate receptors are occurring. In this protocol cellular compartments are sequentially extracted by incubating cells with a number of buffers to separate the cytoplasmic fraction, the membrane fraction, and the nuclear fraction. This gives higher spatial resolution, and furthermore techniques to separate pre and post-synaptic boutons have been developed to identify proteins present in the synaptic cleft (Fabian-Fine *et al.*, 2000).

Is the α7 nAChR responsible for the synaptic changes induced by reinstatement of morphine-CPP?

The putative model outlined above assumes that α7 nAChRs are expressed in the ventral HPC and that they can modulate synaptic plasticity after behavioural treatment.

To remove all concern of unselective effects of MLA, α7nAChR knock out mouse could be evaluated in acquisition, expression, reconsolidation and reinstatement of CPP. Knock out mice with a null mutation of the gene encoding the α7 subunit has been developed (Orr-Urtreger *et al.*, 1997; Marubio & Changeux, 2000). They have been shown to have no α-BGT binding sites and show no sign of rapidly desensitising nicotinic currents in hippocampal brain slice preparations. However these KO mice show behaviour very similar to wild type in cognitive behaviour including the Morris maze task, the Pavlovian conditioned fear test and the pre-pulse inhibition paradigm (Paylor *et al.*, 1998) perhaps due to their absence throughout the brain. Very recently a floxed α7 nAChR conditional knockout has been developed (Hernandez *et al.*, 2014) which allows the selective deletion of α7 from a specific cell type or tissue through genetic manipulation. These mice have an α7 nAChR gene (Chrna7) that is flanked by loxP sites and can be crossed with mice expressing cre recombinase driven by a particular gene, such as dopaminergic genes (Drd2, Drd1), glutamatergic (Grm1-6) or GABAergic (Gabrg1-2) to investigate the effect of

knocking out α7 in different cell types or brain regions. Then the MLA reinstatement experiments could be repeated with these knockout mice to investigate the effect of α7 nAChR in the hippocampus.

Electrophysiology is a powerful tool for exploring changes at a synaptic level. Preliminary work done in the Bailey laboratory shows that MLA (Udakis *et al.*, 2013) and α-BGT (Wright, unpublished data) inhibit LTP in the mPFC and similar effects are seen in ventral hippocampus (Carrrera et al, unpublished data). The role of α7 nAChRs in synaptic plasticity is well documented elsewhere (Broide & Leslie, 1999; McKay *et al.*, 2007) and particularly in the hippocampus (Ji *et al.*, 2001a; Placzek *et al.*, 2010; Yakel, 2012; McQuiston, 2014). Further electrophysiological experiments could be used to shed light on the changes in the hippocampus after in vivo treatment. For example field recordings from *ex-vivo* animals that had undergone CPP could be used to explore the changes in synaptic strength and different levels of the protocol. The floxed α7 nAChR mouse could also be utilised here to investigate changes in either in vitro or in vivo induced plasticity after α7 nAChR knockout.

Why is the α7 nAChR effect specific to reinstatement?

This thesis presents the hypothesis that α7 nAChR plays a specific role in the reinstatement to morphine-CPP but why is the effect specific? As synaptic changes are important for the acquisition and expression of morphine-CPP one would expect the receptors to be important at these stages too. However, the reinstatement trial varies in two ways from the expression test: The reinstatement test is conducted in drugged state, and the reinstatement trial follows extinction training. From this one could hypothesis that α7 nAChRs are essential for the events that occur when a memory is recalled. Extinction has been shown to induce LTP and LTP is know to be required for reinstatement (Portugal, 2014) and consequently blocking α7 receptors within the ventral hippocampus prevents important plastic changes that are required for reinstatement. Support is growing for the idea that cholinergic signalling involves volume transmission, in which diffuse increases in ACh within a brain region modifies the strength of communication (Zoli *et al.*, 1999). Small changes in the ACh, induced for example by a priming dose of morphine and exposure to the drug

paired environment (Fiserová *et al.*, 1999), may be sufficient to shift α7 nAChRs out of their desensitised state into an activate state or *vice versa*.

Furthermore the timing of input of activation from the septal cholinergic input to the hippocampus can induce different forms of plasticity that depend solely on the timing of the input (Yakel, 2012), and this input might vary for cue exposure across the different stages of CPP. Investigation into the changes in acetylcholine release over the course of acquisition, extinction and reinstatement of CPP may reveal an importance for acetylcholine signalling at reinstatement. Acute morphine administration has been shown to decrease glutamate and acetylcholine release in the NAc (Rada *et al.*, 1991; Sepulveda *et al.*, 1998), whilst chronic administration increases glutamate (Farahmandfar *et al.*, 2011b), GABAergic tone (Jolas *et al.*, 2000) and reduces levels of AChE in NAc (Neugebauer *et al.*, 2013). It could be hypothesised that acetylcholine becomes increasingly important as the drug becomes paired with the context, and it is required for establishing the plastic changes that are required for reinstatement. Exploring the changes in acetylcholine throughout the stages of CPP may reveal differences that could support this hypothesis. However determining ACh levels through microdialysis would lack temporal resolution, but Sarter's method for real time measurement of ACh is more effective (Sarter & Kim, 2015). The technique uses enzyme-selective microelectrodes that allow measurements of ACh concentrations with a sub-second resolution (Parikh *et al.*, 2004) With this methodology the role of *in vivo* drug cue exposure could be investigated. Alternatively *in vivo* electrophysiology could be used to investigate activity of the septal cholinergic input into the hippocampus after cue and drug administration.

Do other nAChRs play a role in reinstatement to morphine-CPP?

The data presented in this thesis show that α7 nAChR antagonism significantly inhibits reinstatement but has no effect on the acquisition, expression, or reconsolidation of morphine-CPP. Furthermore mecamylamine had no effect on the acquisition or reinstatement of morphine CPP. There is some evidence that other nAChR receptors are involved. For example Feng et al (2011) showed a role for α4β2 as well as α7 receptors in mediating reinstatement to morphine-CPP, but didn't

investigate this at any other stage of CPP. They showed that the administration of DHβE, an α4β2 selective antagonist, as well as MLA blocked the effect by which morphine priming reinstates morphine induced CPP in BALB/c mice. The broad effect of mecamylamine may be the reason why data here showed no effect on reinstatement. It is generally reported that mecamylamine has preferential affinity for α3β4 receptors versus other nicotinic receptors, for example α4β2 (Papke *et al.*, 2001) and is generally considered to be weaker at α7 (Albuquerque *et al.*, 2009). DHβE, on the other hand, is a competitive antagonist which preferentially binds to β2 containing subunits (Marks *et al.*, 1999), it has specific binding to α4β2 at sub-micomolar affinity but also has affinity at α3β4 and α7 although at 10-50 times lower affinity (Gotti et al, 2006). Therefore it is likely that the effect they see is mediated by α4β2 containing receptors. These receptors have been implicated in studies of learning and memory previously as well as in mediating response to a number of other drugs of abuse (Yeomans & Baptista, 1997; Champtiaux *et al.*, 2006). To test this DHβE could be administered prior to acquisition and to the morphine-priming dose in reinstatement as done for MLA experiments in this thesis.

An effect of mecamylamine on acquisition of morphine-CPP has been shown when it is delivered intracranially into the hippocampus (Rezayof *et al.*, 2006), suggesting that the effects of mecamylamine may be lost with systemic administration. It is possible that mecamylamine may be acting through α7 in this case, due to the different route and dose. To confirm the reinstatement is effect observed in this thesis is due to the α7 nAChR, the reinstatement experiment could be repeated with another antagonist specific to the α7 nAChR such as α-BGT.

Does this finding apply to other drugs of abuse and reinforcement for natural reward?

As drugs of abuse share in parts a common mechanism and nicotinic antagonists inhibit mesolimbic DA release induced by a number of drugs (Zanetti *et al.*, 2006), nAChRs play a role in modulating responses to a number of drugs other than morphine. A4β2 nAChRs have been implicated in methamphetamine (Verrico *et al.*, 2014), cocaine (Guillem & Peoples, 2010) but not alcohol reward (Larsson *et al.*, 2002). Non-selective antagonism of nAChRs has been shown to reduce ethanol

seeking for example both systemic or local (VTA) administration of mecamylamine (Loft et al, 2007) and reduces ethanol seeking (Ericson *et al.*, 1998; Lê *et al.*, 2000; Söderpalm *et al.*, 2000). It has been suggested that this effect is largely dependent on the α6β2* receptors as dihydro-β-erythroidine (DHβE) failed to supress ethanol consumption (Larsson *et al.*, 2002; Kamens *et al.*, 2012). A less comprehensive account for the role α7 nAChRs in other drugs of abuse is available, but it is thought to have a role in reducing 9-tetrahydrocannobonol or cannabinoid-1 receptor agonists' behavioural and neurochemical effects in animal models (Solinas *et al.*, 2007) and cocaine reward (Panagis *et al.*, 2000), but not in reducing ethanol consumption (Kamens & Phillips, 2008).

There is evidence that nAChR modulation of reward responding may be restricted to drug-reward learning. Activation of both muscarinic and nicotinic acetylcholine receptors in the accumbens by ACh volume transmission was necessary for drug conditioning but only muscarinic receptors seem to be important in reward responding for food (Yeomans *et al.*, 1993; Crespo *et al.*, 2006; Sharf & Ranaldi, 2006). Specifically repeated infusions of scopolamine but not mecamylamine, prevent rats from acquiring operant behaviour for food delivery (Sharf *et al* 2006). This dissociation between the ability of mAChR and nAChR to mediate reward could be investigated using the CPP model outlined above, thereby extending this work to look at motivation learning for other rewards. CPP has been utilised to investigate rewards such as sucrose (Agmo *et al.*, 1995), mating behaviours (Paredes, 2009), novelty and social interaction (Ma *et al.*, 2006).

Neuroinflammatory role for α7 nAChRs?

A hypothesis not yet touched on is the idea that this might be an effect of neuroprotection (Shen & Yakel, 2009; Martínez-Hernández *et al.*, 2012) and regulation of inflammation. nAChRs (particularly α7) are expressed on non-neuronal cells in the brain (Wessler & Kirkpatrick, 2008), including astrocytes and microglia (Sharma & Vijayaraghavan, 2002). Lacagnina (2015) found that opioids activate glial cells and alter expression of neuroimmune signalling pathways and reinstatement of morphine-CPP and chemokine gene transcription was blocked with ibudilast (nonspecific glial attenuator). This effect is thought to be mediated through

anti-inflamatory cytokine IL-10 with microglia- methylation at IL-10. Investigate changes in immune signalling after MLA pre-treatment and morphine reinstatement, the floxed α7 nAChR conditional knockout (Hernandez *et al.*, 2014) which allows the selective deletion of α7 from a specific cell type or tissue through genetic manipulation discussed above could be used to knock down α7 nAChRs on glial cells. These mice have a α7 nAChR gene (Chrna7) that is flanked by loxP sites and can be crossed with mice expressing cre recombinase driven by a particular gene, for example glial acidic fibrillary protein (GFAP)- Cre promoter consequently resulting in mice with glial cells that do not express α7 nAChR.

Is MLA interrupting the rewarding properties of the drug of the drug-associated memory?

Is nAChR antagonism disrupting the reinforcement properties of morphine or is it disrupting the recall of the drug-associated cue. For example the morphine may be reinforcing to the animal yet the animals could no long discriminate between the conditioned stimulus and the neutral stimulus. To clarify that the changes are indeed due to changes in the recall mechanism, un-paired morphine administration would have to be investigated. Changes CPP dependent changes in plasticity were not seen when morphine was not paired with a specific context (Portugal et al, 2014). Furthermore we see no effect of MLA on the acquisition of morphine-CPP, which is strong evidence suggesting that MLA does not affect the rewarding properties of the drug but instead the drug-associated memory is affected in reinstatement.

Is state dependency a possible explanation?

A further alternative hypothesis is the idea that MLA is changing the way the animals are experiencing morphine and therefore altering the learned response. State dependent learning is the phenomenon through which memory retrieval is most efficient when an individual is in the same state of consciousness as they were when the memory was acquired. This is very different to the context-dependent driven hypothesis above, which involves the animal's external environment rather than it's internal state. It also strikes some validity with the selective changes seen in the

ventral hippocampus reported in this thesis, as this area has been associated with emotive state (Keralapurath *et al,* 2014).

The data presented within this thesis provides evidence that morphine-CPP is larger during reinstatement than when CPP is measured in a drug free state such as in acquisition. This has been explored experimentally by Zarrindast and Rezayof (2004). They found that mice injected with morphine performed a learned response most efficiently when they were once again under the influence of morphine. It is evident that MLA is not changing the rewarding value of morphine, as it has no effect on the acquisition of morphine-CPP (fig 4.1). However, the MLA reinstatement animals are conditioned with morphine alone, and MLA + morphine at reinstatement may feel sufficiently different, albeit just as rewarding, thereby preventing the expression of the learned response. To assess the effect of state dependent learning, animals could be treated with MLA and morphine during acquisition of CPP then the effect of MLA on the context driven learning could be assessed at reinstatement.

Why is this effect localised to the ventral hippocampus?

This thesis has presented a particular role for the ventral hippocampus in modulating the nicotinic effects on morphine-CPP. Due to the connectivity of the ventral hippocampus (vHPC) with other areas of the reward system and its cholinergic innervation from the medial septum, this area is in a prime position to influence plastic changes occurring in response to morphine-CPP. There is considerable evidence that the vHPC can modulate the neurotransmitter release in mesolimbic areas, such as the VTA (Legault & Wise, 2001; Lisman & Grace, 2005; Valenti *et al.*, 2011). The vHPC is known to project directly to the nucleus accumbens (Christie *et al.*, 1987; Totterdell & Smith, 1989) and activity of the main output of the vHPC, ventral subiculum, is required for novelty induced DA (Legault & Wise, 2001). Furthermore the vHPC projects directly to the mPFC and lesions of the vHPC abolish anticipatory activity in the mPFC (Burton *et al.*, 2009).

α7 nAChRs are present on presynaptic nerve terminals of excitatory neurons to modulate neurotransmitter release, for example in the Schaffer collateral pathway in

the hippocampus, innervating pyramidal neurons in the CA1 region. They are also present on GABA- containing interneurons which make direct contact with pyramidal cells, depending on cholinergic tone nAChRs can either cause disinhibiton or inhibition of these pyramidal neurons, thereby modulating theta rhythm needed for LTP. Therefore the connectivity and expression of α7 nAChR within the ventral hippocampus make it an excellent candidate to modulate synaptic plasticity (Ji *et al.*, 2001a; Fujii & Sumikawa, 2001; McGehee, 2002a; Cobb & Davies, 2005; Maylie & Adelman, 2010).

Does this finding apply to other species?

The work in this thesis suggests that α7 nAChRs play a crucial role in reinstatement to morphine CPP in both mice and rats, but is it relevance to other species? The mouse genome from C57Bl/6J reveals about 30,00 genes, with 99% having direct counterparts in the human genome (Gunter & Dhand, 2002) and this is certainly true for CHRNA7 the gene encoding for α7. Furthermore human α7 mRNA has very similar expression patterns in the human brain, as it is high in the hippocampus, particularly the dentate granular layer and CA2/3 region of the hippocampus as well as the caudate nucleus and the thalamus (Rubboli *et al.*, 1994). It is also well accepted that the fundamental organisation of the hippocampal connectivity, both intrinsic and extrinsic has good agreement across rats, monkeys and humans (Swanson *et al.*, 1978; Witter & Amaral, 1991; Burwell, 2000; Legault & Wise, 2001). There is also evidence that the ventral and dorsal hippocampus serve different purposes in the human brain as they do in rodents (Fanselow & Dong, 2010). Some success has come from targeting nAChR with agonists in the clinic, but antagonising nAChR throughout the brain in humans is likely to have side effect such as impaired cognition.

CHAPTER 7 SUMMARY AND FINAL CONCLUSIONS

The aim of this thesis was to examine the role of nAChRs in motivated reward learning using conditioned place preference focussing on α7 nAChRs. The major findings and conclusions are as follows:

- Pre-treatment with systemic methyllycaconitine (MLA), an α7 nAChR selective antagonist, shows a selective inhibition of reinstatement to morphine-CPP, but not acquisition, expression or reconsolidation in male C57BL/6J mice and Wistar rats.
- Mecamylamine, a non-selective antagonist with low affinity at α7 nAChRs, shows no effect on acquisition of reinstatement to morphine-CPP in male C57BL/6J mice.
- Morphine primed reinstatement significantly increased [^{3}H]AMPA binding in the ventral hippocampus of brain section taken from in vivo-treated male C57BL/6J mice.
- The morphine-induced increase of [^{3}H]AMPA binding in the ventral hippocampus was significantly inhibited by MLA pre-treatment.
- Intracranial infusions of MLA into the ventral hippocampus significantly attenuated reinstatement to morphine-CPP.

In conclusion these experiments provide evidence for the role of α7 nAChR in selectively modulating reinstatement of morphine-CPP. α7 nAChRs in the ventral hippocampus are imperative for this effect, and the changes in [^{3}H]AMPA binding suggest that this is dependent on the receptors role in modulating synaptic plasticity. Further research is required to determine the location of these changes in AMPARs and the levels of endogenous ACh at different stages of the CPP-protocol. Understanding of the associations made between drugs of abuse and contextual stimuli has important implications for the treatments of drug addiction, as preventing relapse in abstaining abusers remains one of the most significant unmet needs in drug addition treatment. In particular morphine induced alterations in hippocampal LTP may have consequences for drug dependence and craving because contextual-driven memories have been associated with drug craving in abstinent drug addicts (Daglish

et al., 2001). Importantly as the mesocorticolimbic brain system did not not evolve to respond to drugs of abuse, examining the neural adaptions elicited by drugs of abuse provides crucial information about changes in the reward circuit and how they contribute to the learning processes involved in normal motivated behaviour.

APPENDIX A - VALIDATION OF MORPHINE CONDITIONED PLACE PREFERENCE

Conditioned place preference (CPP) is an experimental protocol designed to model many types of behaviour including drug-seeking, reinforcement and motivational learning, most commonly in rodents, but also zebra fish (Mustroph *et al.*, 2011), and monkeys (Wang *et al.*, 2011). The CPP apparatus consists of two or three compartments (some boxes contain a neutral section for the unbiased placement of the animal in the box, discussed later) separated by sliding doors to either enclose the animal in either side or allow free movement. The floors and walls of either side of the box vary allowing the animal to differentiate between the two environments and these form neutral cues that become associated with the reward. The animals are enclosed in one side of the box and given a rewarding stimuli and later, in the other side, are given a control treatment. A single CPP-trial has be shown to be sufficient to induce CPP (Mucha *et al.*, 1982) with morphine (Bardo & Neisewander, 1986) and cocaine (dela Cruz *et al.*, 2009) but often >1 pairings with each side occur, one with control and one with the rewarding stimuli. The motivational behaviour is measured by presenting the animal with a choice of environment, either containing the associated cues or the unbiased stimulus, post conditioning. If the treatment is rewarding the animal is more likely to seek the environment associated with it.

Often used to validate a compound's rewarding properties, it is not necessarily a good model of addiction. Rather it is particularly effective at investigating the learning of motivational associations made between a rewarding stimuli and an environment. This is the aspect of the model that will be utilised throughout this thesis.

There are several explanations for what behaviour CPP is actually measuring and it is likely that all outlined below play a part. The first and most popular of these is the incentive-driven behaviour hypothesis and this is thought to rely on the Pavlovian principle. A reinforcer or unconditioned stimulus (US), elicits a pleasurable or desirable effect that the animal is driven to obtain. In CPP this is associated with the visual and tactile cues of the environment and these cues consequently become the conditioned stimulus (CS). Ultimately the CS gains some incentive value of its own and this manifests itself as a place preference. This notion of 'conditioned incentive' (Bolles, 1972; Bindra, 1974) has considerable face validity but it may not be the only

explanation for the phenomenon. Another explanation is that CPP occurs as a result of reinforced behaviour via operant conditioning. The probability of occurrence of any behaviour that resulted or coincided with reinforcer onset will be increased. Therefore when an animal encounters the paired context it is more likely to engage in behaviours that were occurring during the reinforcement and consequently is more likely to remain in the paired context. A good example of this are the psychostimulants where it is well known the behavioural effects are conditionable to environmental cues.

A.1 Considerations in the design of CPP experiments

The general procedure (Rossi & Reid, 1976) described above remains largely unchanged since the early studies done by Rossi and Reid (1976), however there are certain parameters that can be modified to optimise for certain objectives.

Apparatus

An important initial consideration is whether the apparatus is designed in a way that forms little animal preference for either environment (unbiased design), or in a way that the animals show an unconditioned preference (biased design) to the apparatus. Due to the nature of the paradigm bias is often unavoidable. Frequently tactile stimuli are favoured over visual cues as the conditioned stimulus as they appear to form less preference bias. Smooth floors and white versus black walls have been observed to cause bias, as animals prefer dark environments with textured floors (Cordery *et al,* unpublished observation). Another problem is which side the experimenter places the animal in at the beginning of the experimental trial. If an animal is particularly stressed it may just remain in this first environment rather than exploring to find the preferred side. The apparatus is often designed to account for this by incorporating a neutral centre compartment where the animal is placed by the experimenter. However this can complicate analysis of the data (discussed later), especially if animals favour this neutral area.

Unconditioned stimulus

Opiates, such as morphine and heroin are capable of producing CPP in rats and mice due to their ability to induce release of dopamine from mesolimbic DA neurons. μ-opioid receptors on GABAergic neurons in the ventral tegmental area (VTA) inhibit GABA release, and therefore GABAergic inhibition of dopaminergic cells is reduced (Johnson & North, 1992). A wealth of literature indicates morphine, a μ-opioid receptor agonist, is rewarding when administered systemically to animals (Narita *et al.*, 2001). This has been shown to be dependent on DA release as morphine CPP can be blocked by D_2 receptor antagonists (Manzanedo *et al.*, 2001).

Experimental subjects

Another primary decision is the type of animal to be used in any behavioural research. In this thesis the work is limited to rodents as a result of the trade off between the genetic, biological and behavioural similarity to humans and the convenience and ethical considerations of the research. Traditionally CPP research was conducted in the rat, but in the last two decades advances in transgenic science has seen an increase in the use of knock out mice in such studies.

In this thesis all systemic behavioural work is conducted in mice to investigate the effects of nicotinic acetylcholine drugs on CPP. Selecting a strain of mouse is complicated by the fact that the literature reports variable susceptibility to CPP in different mouse strains. The C57BL/6 (B6) is the most commonly used mouse strain in behavioural studies (as shown in table A1) due to its apparent sensitivity to rewarding agents showing clear place preference in comparison to other strains. Although success has been seen in other mouse strains, for example across six commonly used laboratory strains (C57BL/6L, BALB/cJ, C3H/HeJ, DBA/2J, FVB,NJ, 129S1/SvlmJ) cocaine-induced CPP was observed in all strains with the exception of D2 and 129 (Eisener-Dorman *et al.*, 2011). This variable susceptibility to CPP in mice perhaps is to be expected when we consider the variability of human behaviour. Furthermore it can be argued that there is growing agreement that genetic factors play an important role in sensitivity to drug dependency and addiction in both humans and animals (Bailey *et al.*, 2010).

Age of the animals is an important consideration as it is well established that the adolescent brain is differentially sensitive to drugs of abuse like nicotine (Belluzzi *et al.*, 2004). Although Laviola *et al* (1992) studied the ontogenetic pattern of cocaine reinforcement in outbred CD1 mice and found that place preference was inducible, with the highest dose; in all three age groups (14-17, 21-24, or 28-31 days) they found differences in the sensitivity to cocaine. For example 5mg/kg dose was only effective in inducing CPP in the 21-24 day old mice (Laviola *et al.*, 1992). Although tested in a biased chamber set up (a black and white chamber) the drug experience was paired with the least favourite chamber therefore results should be relatively robust. No sex differences were found although nearly all studies use male mice. There are some examples of female mice in CPP studies that have attempted to model human social pressures that may influence relapse (Mattson & Morrell, 2005).

Appendix A- 1 The strain and age of mice used in CPP

Paper	Strain	Comment?	Age (~weeks)
(Cunningham *et al.*, 2006)	DBA/2J C57BL/6J	C57BL/6J CPP appears to be unaffected by trial length	8-10
(Dong *et al.*, 2004)	C57BL/6		3-4
(Eisener-Dorman *et al.*, 2011)	C57BL/6L, BALB/cJ, C3H/HeJ, DBA/2J, FVB,NJ, 129S1/SvlmJ	CPP observed in all but DBA/2J and 129S1/SvimJ	8-10
(Feng *et al.*, 2011)	BALB/c		5
(Grabus *et al.*, 2006)	C57BL/6, DBA/2J	Nicotine induced CPP seen in C57BL/6 only	10
(Heinrichs *et al.*, 2010)	C57BL/6	Sensitivity to opioids	9
(Laviola *et al.*, 1992)	CD1	CPP in all age groups with 25, and 5mg/kg only showed results in 3-4 week age group	2- 3, 3-4, 4-5
(Risinger & Oakes, 1995)	Swiss-Webster		8
(Schlussman *et al.*, 2008)	C57BL/6J and 129P3/J	129P3/J less sensitive to heroin	6
(Shoblock *et al.*, 2005)	C57BL/6		8-10
(Walters *et al.*, 2006)	C57BL/6		8

As the hypothesis of the thesis progresses, for example into intra-cranial infusions the need to extend the work to larger more complex organisms will arise. Rats make excellent subjects for the study of intracranial infusions due to their robust nature and relatively large size. Male Wistar rats are often selected for such studies due to their docile nature (Harlan) and a University of Bath breeding colony offers the advantage of not having to transport the animals.

Experimental protocol

The experimental protocol has many variations and below these variables are discussed and settled for use throughout the rest of this thesis.

Handling and Habituation

It is common practice to allow the animals to explore the experimental apparatus prior to conditioning to control for the effect of novelty. Due to the stressful nature of experimenter handling, simply releasing an unhandled animal into a chamber may have reward effects of its own. As a consequence this may mask the effects attributed to the pharmacological treatment. During this exploratory phase the animals are allowed access to both compartments of the apparatus for around 15 minutes. The time spent in each compartment is recorded and this allows any initial preference to be measured. Often if animals show a strong preference for a particular environment (for example more than 10 minutes in any one compartment) then they are excluded from the study. It is argued by some that this first 15 minutes represents exploration rather than preference. Bozarth (1987) has tested the stability of the preconditioning preference test, which is critical if the shift in preference is to represent the effect of drug-paired conditioning. Although there is a trend to the non-preferred side it is not significant and initially stabilises after two sessions (Bozarth, 1987). This justifies the use of both an exploratory session in addition to the pre-conditioning preference test (both 15 minutes) or alternatively one pre-test session of 30 minutes. The effect of repeated apparatus exposure will be investigated to determine which method will be used throughout this thesis.

Conditioning

Another consideration when utilising CPP is the number of conditioning trials required to form reliable conditioning. Bozarth (1987) tested the effect of conditioning trials and found little benefit, in terms of enhanced conditioned response, after 3 conditioning trials. In our laboratory CPP experiments normally consist of two drug-paired trials (CS+, where the drug is paired with one floor cue) and two vehicle-paired trials (CS-, where saline is paired with one floor cue). Although stronger conditioning may be achieved by more trials, this might not be a time efficient increase (Bardo & Bevins, 2000).

Temporal parameters of conditioning protocols also differ between different researchers; some prefer to leave 24 hours between successive conditioning trials while others conduct two conditioning trials per day. There are two potential problems with the latter method: the drug can become associated with the time of day rather than the floor cue, and the consolidation period for the morning trial is interrupted by the second (afternoon) trial. Although a significant time period is left between the two trials animals in drug-paired chambers in the afternoon will have longer to consolidate what has been learnt without the disruption of another trial. These problems can be avoided by a cross-over design, which can be completed in 4 days rather than 10. With this procedure the drug and control are each paired with both floor cues and both times of day across the animal groups, therefore animals that received saline in the morning session will receive morphine in the afternoon.

The time interval between the administration of the US and the exposure to the CS has been shown to influence the strength of CPP, although more strongly in certain strains of mice. Ideally blood and brain drug levels should be rising during the initial part of the trial. Cunningham *et al* (2006) found that a five-minute trial time was sufficient to induce CPP in DBA/2J mice when using intraperitoneal ethanol, but when using cocaine a longer trial duration (~60mins) is necessary. With morphine-CPP it has been found that either short (30minutes) or longer session (40-45minute) sessions give a more robust CPP (Bardo *et al.*, 1995) Consequently the mode of delivery is an important factor influencing the strength of CPP, as drugs dosed *per*

oral will take longer to be absorbed in comparison to intraperitoneal delivery. Furthermore the optimal trial length is thought to be around 30 minutes, although not significant from 10 minutes (Billa *et al.*, 2009). These temporal factors may be strain specific and consequently may not be optimal for all strains. Certainly though, a balance needs to be sought between time efficiency and gain in terms of strength of CPP, as investment of time may make only a very small change to the strength of CPP.

Post-test

24 hours post-conditioning the conditioned preference is tested with a 15-20 minute trial where the animals are allowed to choose between the CS+ and CS- by removing the central doors. This is mostly done by counter balancing the left/right position of the cue within each group to control for any exogenous biases within the experimental room (for example external noise or draft). Some studies favour administering an injection of vehicle prior to the test to avoid bias from the novelty of experimental procedure (Bardo & Bevins, 2000), while others favour no interference before testing (Feng *et al,* 2011). The effect of this was out of the scope for this thesis, therefore no interference before testing was practised during all experiments conducted in this thesis.

Maintenance and Extinction

There are two widely accepted methods of inducing extinction: explicit or un-explicit paired training. Explicit paired training (EP) involves pairing a previous drug paired environment with the absence of the drug normally by saline injection. While un-explicit paired training (UP) involves passive exposure to drug-paired environment by allowing animals access to both environments without drug reward. The UP procedure takes longer, for example Feng *et al* (2011) conducted extinction training for 7 days without injections compared to only 4 days in EP paradigms. It should be noted that UP has more validity as a model of human relapse, as it relies on contact with the CS which is more typical in human behaviour (Brenhouse *et al.*, 2010). The common criterion for extinction is when an animal spends <55% in the drug paired side for two consecutive days (Shoblock *et al.*, 2005; Billa *et al.*, 2009). Typically animals that do not meet this criterion are excluded from further training.

Reinstatement

CPP can be used as a model of relapse triggered by exposure to cues associated with the drug. It has been used in many studies and is widely accepted as a laboratory correlate of relapse to drug seeking (Do Couto *et al.*, 2003; Ribeiro Do Couto *et al.*, 2005a; Quirk & Mueller, 2009; Aguilar *et al.*, 2009; Feng *et al.*, 2011). Reinstatement models relapse by prompting drug seeking behaviour with a priming dose of morphine (Do Couto *et al.*, 2003; Popik *et al.*, 2006), or cocaine (Mueller & Stewart, 2000) then animals are allowed free access to both compartments for 15 minutes. The idea that re-exposure to drug after extinction of CPP is sufficient to cause relapse is well established (Mueller & Stewart, 2000; Wang *et al.*, 2000; Manzanedo *et al.*, 2001; Itzhak & Martin, 2002)**.** However there is also a growing body of evidence suggesting that exposure to environmental drug associated cues (Lu *et al.*, 2002) and/or stress (Wang *et al.*, 2000; Sanchez & Sorg, 2001) also play important roles in relapse.

A.2 Aims

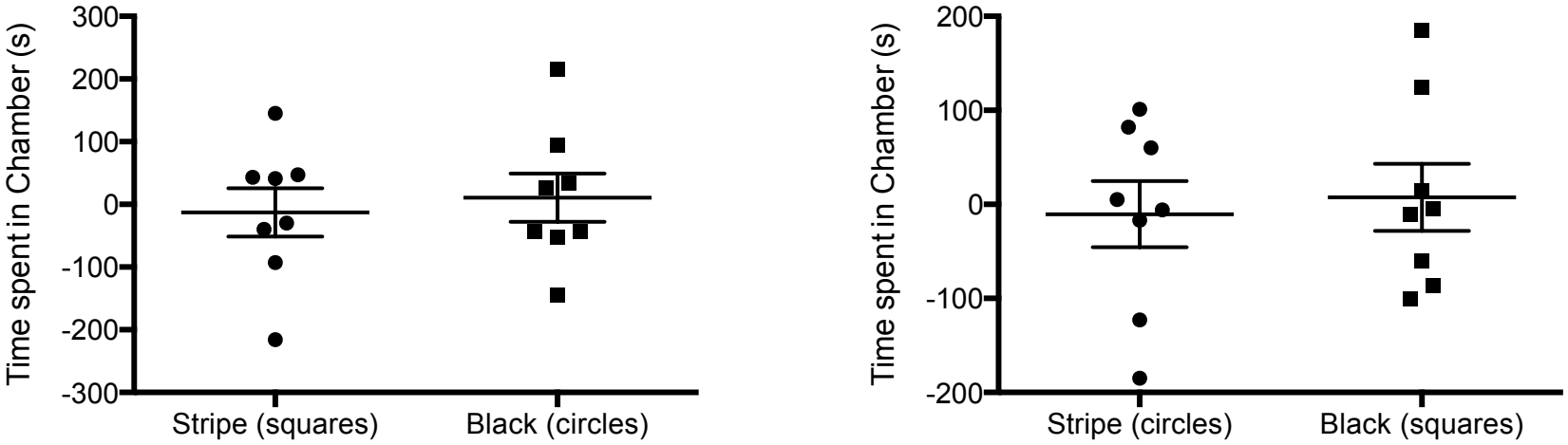
The research documented in this chapter was aimed at exploring and validating some of the parametric aspects of morphine-CPP. To explore the parameters outlined above several experiments were conducted to establish a balanced apparatus-based protocol, including a series of experiments to optimise acquisition of morphine-CPP. The effects of prior handling and noise during the conditioning trial were investigated, as stress appears to adversely affect the establishment of morphine CPP. Finally protocol parameters such as number of conditioning trials per day and a comparison of morphine and saline pairings were also optimised.

A. 2 Results

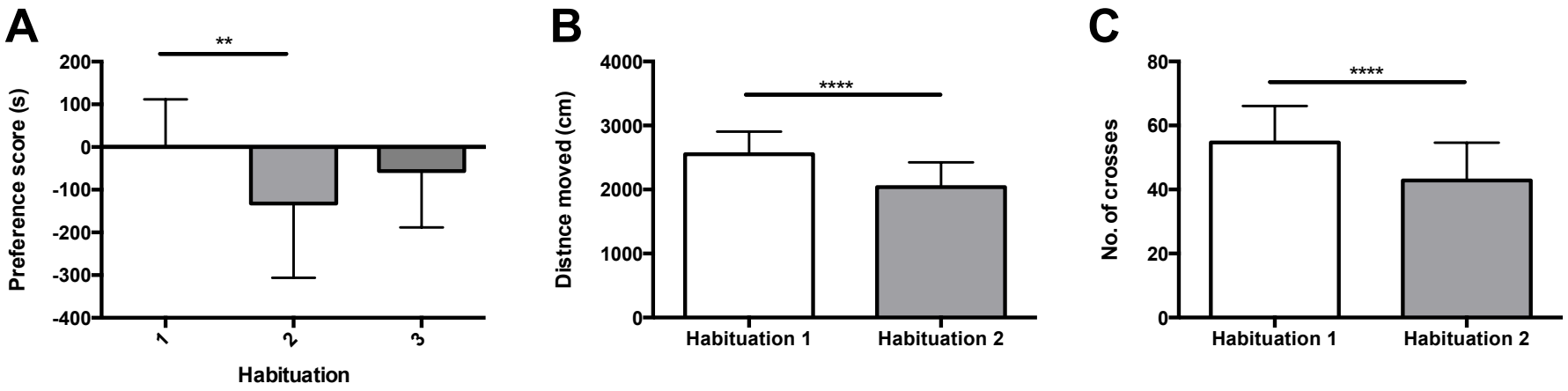
Establishing a balanced CPP protocol

A small pilot study was conducted to test the effect of different floor pairings (either small circles or small squares) with the two contexts (either black or striped). Naïve C57 mice were placed in the CPP apparatus for 2 habituation sessions (15mins), and the amount of time they spent on each side was recorded. Data revealed no statistically significant difference in the time spent in each side for both combinations of cues and floor contexts. The circle floor context (2mm holes, 3mm interax) in the black side and square floors context (4x4 holes, 7mm interax) in the stripy (the configuration used in figure A2) was used for future experiments.

The stability of the preference test was also examined, which is critical if a shift of preference is taken to represent drug-paired conditioning (Wise & Bozarth, 1987). If preference significantly changes in between the habituation and conditioning trial independently from the drug conditioning then this will be wrong attributed to a conditioned effect. Naïve mice were placed into the CPP apparatus for 15 minutes on three consecutive days. The data in figure A.3A shows that the time spent in the least preferred side after the first habituation significantly reduces after the first trial (one-way ANOVA $F(2,69)=5.260$, $p=0.0075$, Dunnet's *post hoc* revealed $p=0.0037$) and stabilises towards the original preference by the third showing no statistical significance from habituation 1 ($p=0.2930$). This therefore justifies the use of two 15-minute habituation tests before the conditioning begins if change in preference is to be dependent on drug-paired conditioning. The distance moved (paired t-test $p=0.0001$, Fig. A.3B) and the number of crosses (paired t-test $p=0.0001$, Fig. A.3C) through the middle also significantly reduced after the first habituation test.



Appendix A- 2 Establishing a balanced protocol.
Eight Naïve male C57BL6J mice were placed in the CPP apparatus and the time they spent in either the black or the striped chamber was recorded with ethovision software. The removable floor contexts (either circles or squares) were switched to establish the most balanced combination.



Appendix A- 3 Stability of preference.

A) The time spent in the least preferred side significantly reduces and stabilises after 2 trials. Eight Naïve male C57BL6J mice were placed in the CPP apparatus 3 times and the time they spent in the least preferred side was recorded with ethovision software. The time spent in the least preferred side significantly reduced after the first trail but stabilises after 3 (ANOVA with repeated measures $F_{(2,69)}=5.260$, $p=0.0075$, Dunnet's *post hoc* revealed $p=0.0037$). The distance moved (B, paired t-test p=0.0001) and the number of crosses (C, paired t-test p=0.0001) also significantly reduces after habituation one.

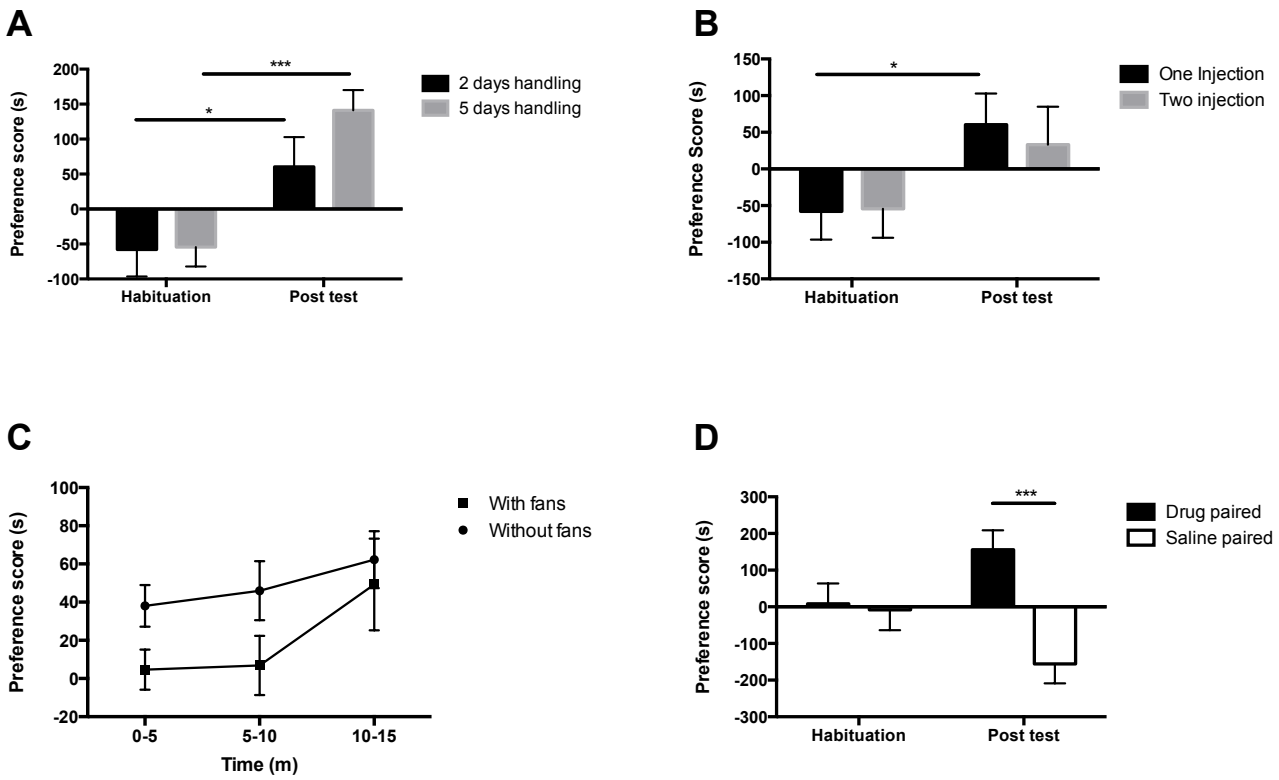
Optimising acquisition of morphine-CPP

A number of steps were taken to optimise both the protocol and the apparatus, to maximise morphine-CPP. First, the effects of increasing the number of days of handling from two to five days were compared. Five days of handling increased the time spent in the drug-paired chamber from 60±42s to 141±29s post conditioning.

The fans within the sound attenuating chambers where also assessed for their effect on the development of CPP. Mice were conditioned with the fans either turned on and off and the effect was recorded by observing the time spent in the drug-paired chamber after morphine pairing. The data reveal that the fans negatively impacted on the expression of CPP Fig A.4C. In the first time bin the difference was the largest 4.7±10s compared to 38±10s without the fans.

Figure A.4 shows the effect of 2 injections per day compared to only one. Increasing the number of conditioning trials per day seemed to have little effect on the measure of conditioned place preference but significant CPP was only observed in the one injection group. This suggests that 2 injections a day were less effective or at least more variable.

To test whether saline pairings on both sides of the apparatus would change total time spent in the any one side, animals were either paired with morphine on consecutive days (10mg/kg, i.p) or saline every day. Figure A.4D shows that if both compartments were paired with saline during the conditioning phase the amount of time spent in either side was not significantly changed.

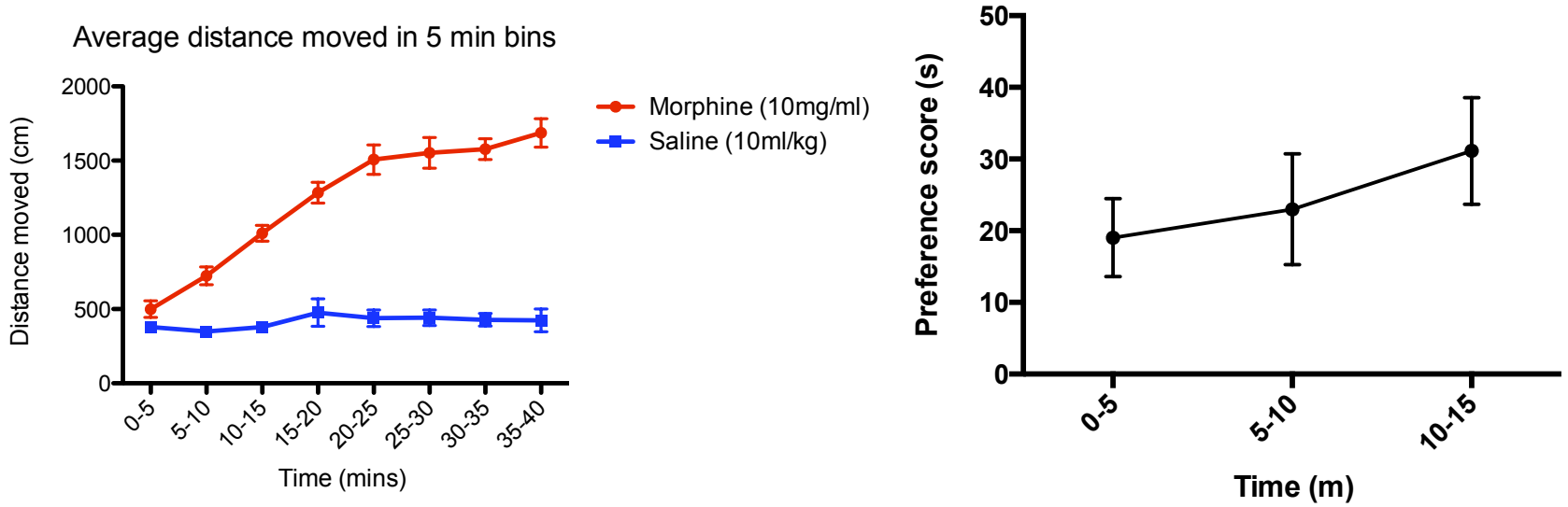


Appendix A- 4 Optimising CPP.

A) Effect of handling. Animals were handled for 2 (black bars) or 5 (grey bars) days before being introduced to the morphine-CPP (10mg/kg, i.p) and scored for time spent in drug -aired chamber. Animals handled for 5 days show greater CPP than those handled for 2 (One-way RM ANOVA, **p*=0.003,****p*=<0.001 n=12/treatment) . **B)** Effect on number of conditioning trials. Animals were either given 1 or two injections a day for 4 days of conditioning (10mg/kg, i.p) and scored for their time spent in the drug paired side. There is no significant difference between one or two conditioning doses/ day (One-way ANOVA,**p*=). **C)** The effect of fan noise. Conditioning trials were either conducted with or without the fans. The effect of the fans within the sound attenuation boxes on the expression of morphine-CPP shown in 5 min time bins (one-Way ANOVA p=0.156, 0.098,0.578). **D)** The effect of morphine and saline conditioning on CPP. Animals were paired either with only saline on both sides of the chamber or morphine and saline on consecutive days.

Trial time

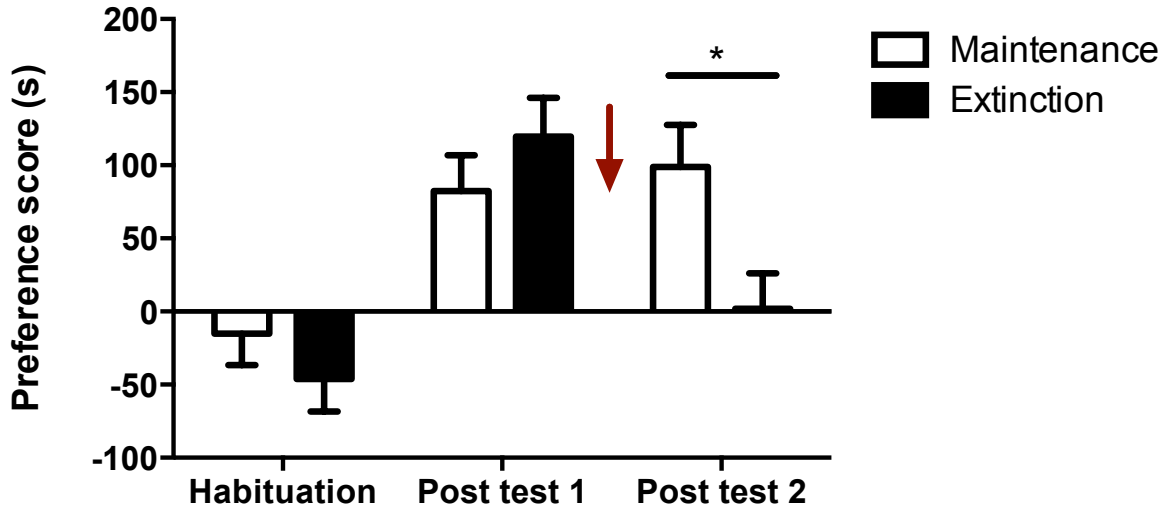
To investigate the ideal length of the conditioning trial locomotor activity was recorded over 40 minutes in a drug and saline-paired animal. No change is seen in locomotor activity in animals treated with saline. Morphine increases locomotor activity over 40 minutes. The psychomotor stimulant theory of addiction (Wise and Bozarth, 1987) defines psychomotor stimulant properties of rewarding drugs as predictors of whether the drug will prove rewarding. Morphine acts as a locomotor stimulant in C57BL6J mice (Oliverio & Castellano, 1974) and there is a case for using this characteristic as an indication of drug longevity in the drug conditioning trial (figure A.5). This might explain why data shown later shows better CPP expression during the second half of the reinstatement trial, as the subjective pleasurable effect may also not be at the maximal level until approximately 15 minutes post-injection (see reinstatement).



Appendix A- 5 Experiments validating trial time.
A) The graph shows the distance moved in 5-minute time bins over the 40 minute trial for saline (10ml/kg, i.p) and morphine (10mg/kg, i.p) treated animals. No change is seen in locomotor activity in animals treated with saline. Morphine increases locomotor activity over 40 minutes. B) The expression of preference over the 15 minute post test trial. Preferecne for the drug-paired side increases over the 15 minute trial time.

Maintenance and extinction of morphine CPP

To investigate whether this increased time spent in the drug paired (DP) chamber post conditioning is maintained in the absence of the unconditioned stimulus (morphine), animals were left undisturbed in their holding cages for 6 days, before being tested again for preference (day 16). Figure A.6 shows a significant effect of test ($F_{(2,140)}$=18.61, p=<0.001) but not of treatment ($F_{(1,70)}$=1.55, p=0.217). Pairwise comparisons revealed no difference at habituation test (Maintenance: -15.1±21.5, extinction: -46.1±22.3s in drug paired side, p=0.377, n=36), or post test (Maintenance: 82.4±24.4s, extinction: 119.6±26.5s in drug paired side, p=0.289, n=36). When these animals were tested again on day 16, maintenance of morphine-CPP was observed only in the maintenance group. Animals that underwent repeated saline pairing with both chambers for four days showed no maintenance of morphine CPP and these were significantly difference from the maintenance group (Maintenance: -98.95±28.7, extinction: 1.8±24.28s in drug paired side, p=0.018, n=36).



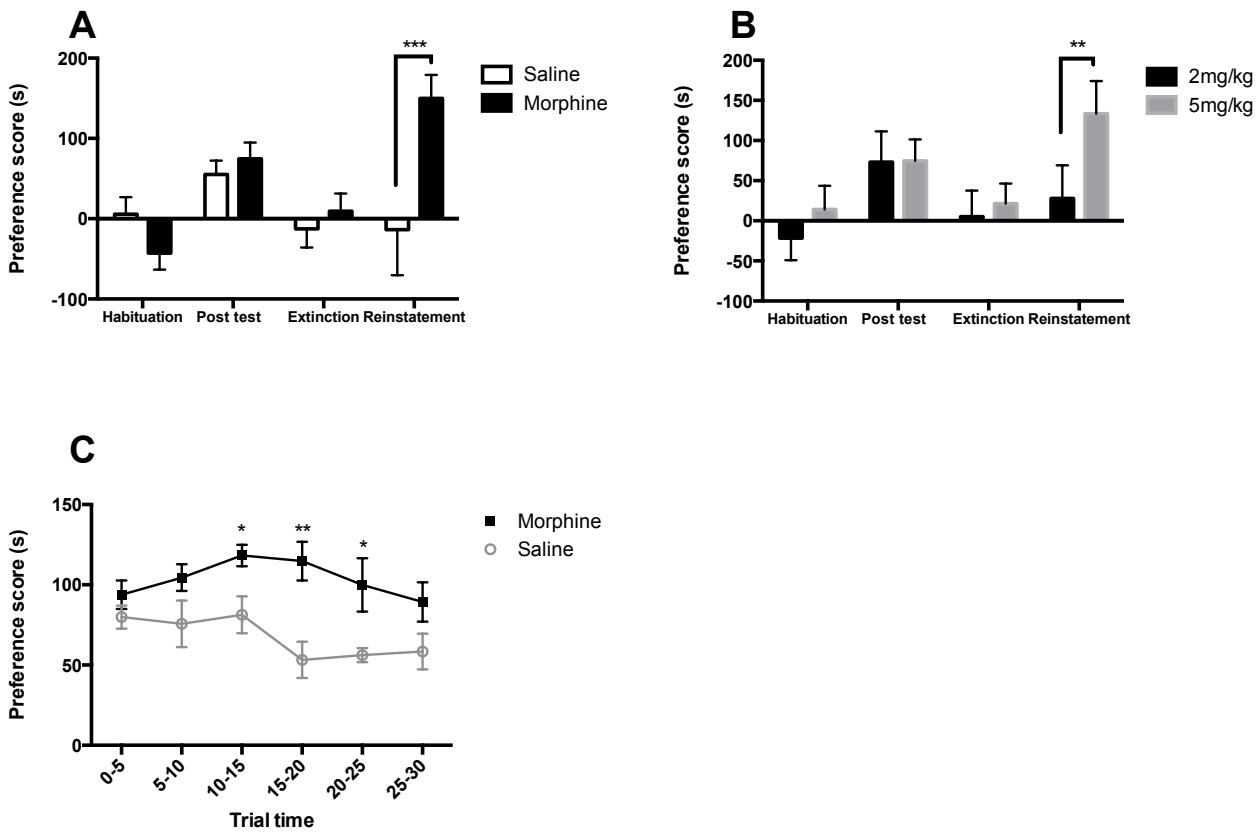
Appendix A- 6 The maintenance and extinction of morphine-CPP.
After acquisition of morphine-CPP animals were either left undisturbed in their home cages for 6 days (maintenance group) or underwent EP extinction training (extinction group). CPP was observed only in the maintenance group. Animals that underwent repeated saline pairing with both chambers for four days showed no maintenance of morphine CPP and these were significantly different from the maintenance group (Maintenance: -98.95±28.7, extinction: 1.8±24.28s in drug paired side, p=0.018, n=36).

Reinstatement

To investigate the effect of reintroducing the unconditioned stimulus after extinction, mice that reached the criteria for extinction (see methods Chapter 2.) were selected for the reinstatement test. A preliminary study was done to investigate the effect of different priming doses on reinstatement (figure A.7B). Animals were either treated with 2mg/kg or 5mg/kg priming dose of morphine, as it has been shown that reinstatement priming doses lower than the training dose are effective (Do Couto *et al.*, 2003) A repeated measures one-way ANOVA showed that there was a non-significant effect of treatment ($F_{(1,14)}$=1.72, p=0.211) but a significant effect of test ($F_{(3,42)}$=1.24, p=<0.011). *Post-hoc* comparisons revealed significant difference between reinstatement in the two treatments (morphine 2mg/kg: 55.9 ±82.2s in drug paired side, morphine 5mg/kg: 267.0±81.2s in drug paired side, p=0.03, n=8/treatment group).

In subsequent studies a priming dose of either morphine (5mg/kg, i.p) or saline (10ml/kg, i.p) was given prior to a final 30-minute exploratory trial (figure A.7A). A repeated measures one-way ANOVA shows that there was a non significant effect of treatment ($F_{(1,20)}$=3.12, p=0.093) but a significant effect of test ($F_{(3,60)}$=5.47, p=0.001). The was a significant interaction between the two factors $F_{(3,60)}$=5.47, p=0.002, n=10-12/treatment). *Post-hoc* comparisons revealed significant difference between reinstatement in the two treatments (morphine: 300.0 ±58.3s in drug paired side, saline: -27.4±133.1s in drug paired side, p=<0.001, n=10-12/treatment group.)

The time course of the expression of reinstatement was investigated by looking at the expression of reinstatement over 5 minute time bins (figure A.7C). Multiple t-tests (false discovery rate set at 1%) showed significance at 10-15 (p=0.03), 15-20 (p=0.009), and 20-25 minutes (p=0.04).



Appendix A- 7 **Reinstatement of morphine-CPP by morphine drug priming.**

A) After acquisition and extinction training a priming dose of either morphine (5mg/kg, i.p) or saline (10ml/kg, i.p) was given prior to a final 30-minute exploratory trial. Only the morphine treated group reinstated (morphine: 300.0 ±58.3s in drug paired side, saline: -27.4±133.1s in drug paired side, p=<0.001, n=10-12/treatment group. B) the effect of varying the priming dose was investigated on reinstatement. Only the 5mg/kg group reinstated and this was significantly different to the time spent in the drug paired side with 2mg/kg treatment group. C) The time course of the expression of reinstatement was investigated by looking at the expression of reinstatement over 5 minute time bins. Multiple t-tests (false discovery rate set at 1%) showed significance at 10-15 (p=0.03), 15-20 (p=0.009), and 20-25 minutes (p=0.04).

A.3 Discussion

In this chapter an unbiased, 2 chamber, CPP protocol has been established in C57BL/6J mice 6-8 weeks old. The validation studies revealed no significant preference to either side and consequently for all subsequent experiments animals were pseudorandomised so that the same numbers were drug-paired to the black and striped side. Two habituation tests were chosen as the time spent in the least preferred side stabilised after two sessions and an average was used as the baseline. A protocol with 4 x 40 minute acquisition sessions was conducted in the sound attenuation boxes with the fans turned off. 10mg/kg (1 mg/ml) morphine and 10ml/kg saline were delivered i.p on subsequent days. Explicit extinction training was shown to significantly extinguish morphine-CPP expression compared to simply the passage of time, and a priming dose of 5mg/kg, i.p (half the conditioning dose) was selected as the optimal dose for reinstatement and the second 15 minute time bin was selected as a more reliable measure of CPP.

The data collected in the stability of the preference test confirms that shift in preference is likely to represent drug paired conditioning. Similar to Wise and Bozarth (1987**)** we found a non-significant trend towards the non-preferred side which stabilises after two trials. It has been argued that the first 15-minute trial represents exploration rather than true preference. These findings justify the use of two 15 minute habitation tests and taking an average of the two to form the base line.

The balancing data show that pairing the striped arena with the squared floor and the black arena with the circled floor provides a balanced combination, although it must be acknowledged that the floor pairing had little significant effect on preference perhaps reinforcing the importance of visual cues in the conditioning paradigm. Additionally this justifies the use of low-level white light during the experimental procedure, which Cunningham & Zerizef (2014) found critical for the expression of CPP. Although it is often assumed that cues from several modalities is better than one, for example visual and tactile cues. Caution should be exercised when drawing conclusions from this balancing data as animals may have become habituated to the apparatus.

This work demonstrates that a morphine-induced CPP can be acquired, maintained for one week after conditioning training, extinguished then reinstated by the non-contingent administration of morphine. Several studies have shown that CPP can be maintained for extended periods of time in mice. For example Mueller et al (2002) have reported that morphine-CPP can be maintained for up to 12 weeks. In agreement with previous models of extinction, this protocol used here readily induces extinction of CPP (Mueller et al, 2002). Explicit daily extinction trials diminish the CPP, implying that the CS environment associated with the drug become less salient after repeated pairings with saline. The data shown here, and from other laboratories (Mueller *et al.*, 2002; Sakoori & Murphy, 2005), confirm that in the absence of explicit extinction training, drug associated CS+ are expected to maintain their ability to promote drug-seeking behaviour. Studies have shown that EP and UP training paradigms are equally efficient for extinguishing conditioned place preference, although stronger reinstatement is shown in EP trained adult rats.

We found that, after extinction, a single non-contingent injection of morphine (5mg/kg, i.p) reinstates morphine-induced CPP, as measured by increased time spent in the drug-paired chamber. This is consistently stronger than the CPP seen after acquisition and may be due to the effects of state-dependent learning, the idea that memory formed in a particular state can only be retrieved or reproduced when the animal is in the same state (Overton, 1978; Weingartner, 1978)**.** The possibility of this phenomenon happening here are obvious as the memory is acquired under the influence of morphine but tested in a drug-free state after acquisition but drug primed for reinstatement. We find that 2mg/kg morphine was an insufficient priming dose to reinstate morphine CPP. This finding is in line with other studies that find 2 or 3mg/kg morphine is insufficient to reinstate morphine CPP (Ribeiro Do Couto *et al.*, 2005b).

Conclusions

These experiments revealed that:

1) Repeated exposure to the CPP apparatus shows a non significant trend towards the non preferred side which stabilises after two trials.

2) Balancing data showed that pairing the striped arena with the squared floor and the black arena with the circled floor provided a balanced combination for pseudorandomisised trials.

3) This work demonstrates that a morphine-induced CPP can be acquired, maintained for one week after conditioning training, extinguished using EP pairing training.

4) The reinstatement of CPP can be induced with 5mg/kg morphine but not 2mg/kg in C57BL6/J mice.

Thus the CPP apparatus and procedure has been optimised and validated for morphine CPP, in preparation for evaluation of the role of nicotinic receptors in this motivational learning paradigm. An unbiased two-chamber model, with the black context paired with circle floor and the striped context paired with the square floor, was used in a one-injection per day, crossover design paradigm. C57BL6J mice were handled for 5 day prior to 2 habituation sessions (15minutes), then conditioned with morphine (10mg/kg) and saline (10ml/kg) on alternative days for 4 days, before a final preference test (15 minutes). Extinction was conducted in a EP manner with saline given on both sides on consecutive days, before a reinstatement test (30 minutes) where a drug priming dose (5mg/kg, i.p) was given immediately before entry into the box.

APPENDIX B - THE EFFECT OF SOCIAL HIERACHY ON CPP

Housing and social factors

The vast majority of behavioural research is conducted in same sex animals housed in groups to avoid behavioural complications such as courting, mating and fighting for a mate. However, mice are territorial animals and it is know that even in the absence of females cage hierarchy exists and may have important implications on subsequent behavioural experiments. There is evidence that the social hierarchy within a cage as may impact on responses to rewarding stimuli. The tendency of an animal to make a goal response can be affected when this response involves overcoming the competing response of another animal that is similarly motivated (Lindzey *et al.*, 1961). In group-housed mice, dominance can be determined using the social dominance (tube) test and is routinely used in phenotyping of newly-derived transgenic mouse lines (Lindzey *et al.*, 1961); The social dominance tube test can measure aggressive tendencies in mice without exposing the animals to physical injury. The animals are inserted and released into either end of the tube simultaneously and the mice are scored for submissive or dominant behaviours in each sort trial. The dominant mouse generally forces the subordinate out of the neutral centre area. A lack cocaine reinforcement has been reported in monkeys (Morgan *et al.*, 2002) and rodents (Schenk *et al.*, 1987; Lesage *et al.*, 1999). Social defeat in dominant male rats has been associated with a decrease in consumption of palatable sucrose solution and a loss of cocaine induced CPP (Riga *et al.*, 2015), and exposure to rats of similar age and weight has been shown to reverse acquisition and reinstatement of cocaine induced CPP (Fritz *et al.*, 2011).

B.1 Methodology

Social dominance test

Mice (32 male C57BL/6J) were gently restrained at either end of the tube (a 30cm perplex tube; the diameter of the tube was just sufficient to allow a mature mouse to pass through it, but not wide enough to permit two mice to pass one another). The mice were then simultaneously released to explore in a forward direction. A mouse deemed dominant if it approached, whilst the subordinate backed away, forcing the subordinate out of the tube. The trial ended when one mouse had forced the other to retreat with all four feet out of the tube. Each animal was paired with each of its 3 cage mates and each match was repeated twice. The animals were determined to be have 'alpha' dominance if they were they displayed dominant behaviour in each of the pairings with their cage mates. 'Beta' mice only submitted in pairings with the dominant (alpha) mouse and subordinate mice submitted to both the alpha and beta mouse in the cage. To test the effect of this social hierarchy on morphine-CPP all animals then underwent CPP training, extinction and reinstatement. At the end of the experiment animals were killed by cervical dislocation.

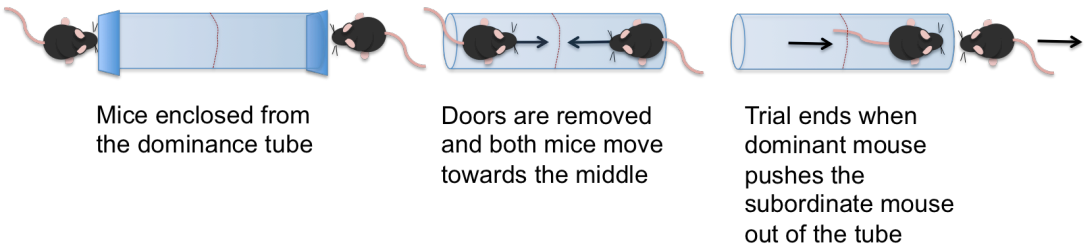


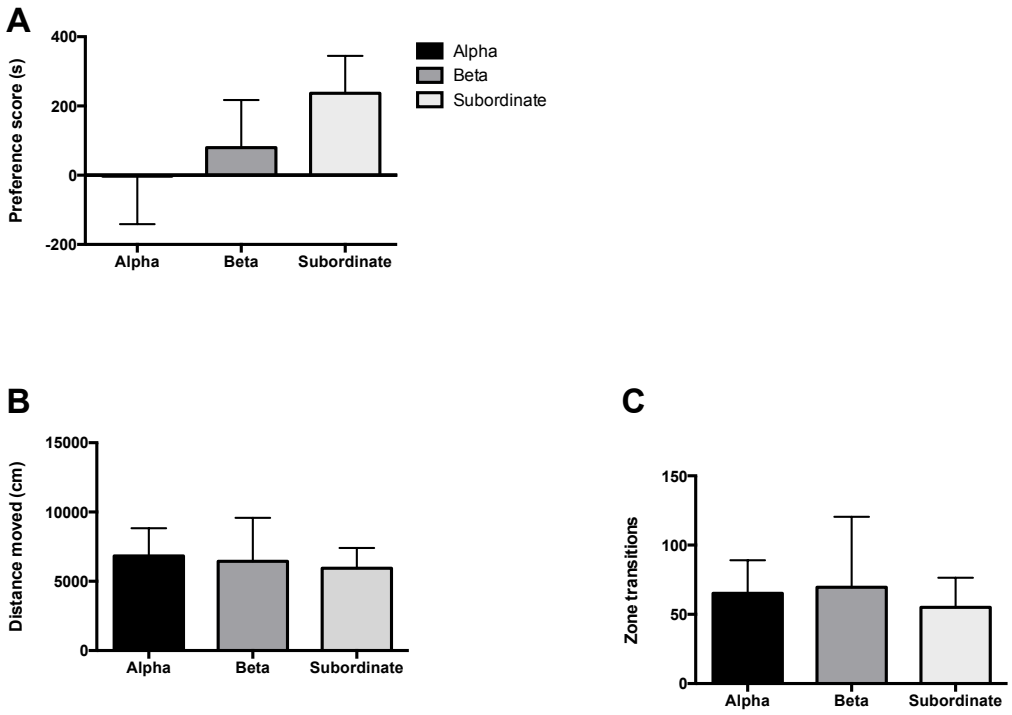
Figure B-1 A schematic showing the protocol for the social dominance test

B.2 Results

The effect of social hierarchy on CPP

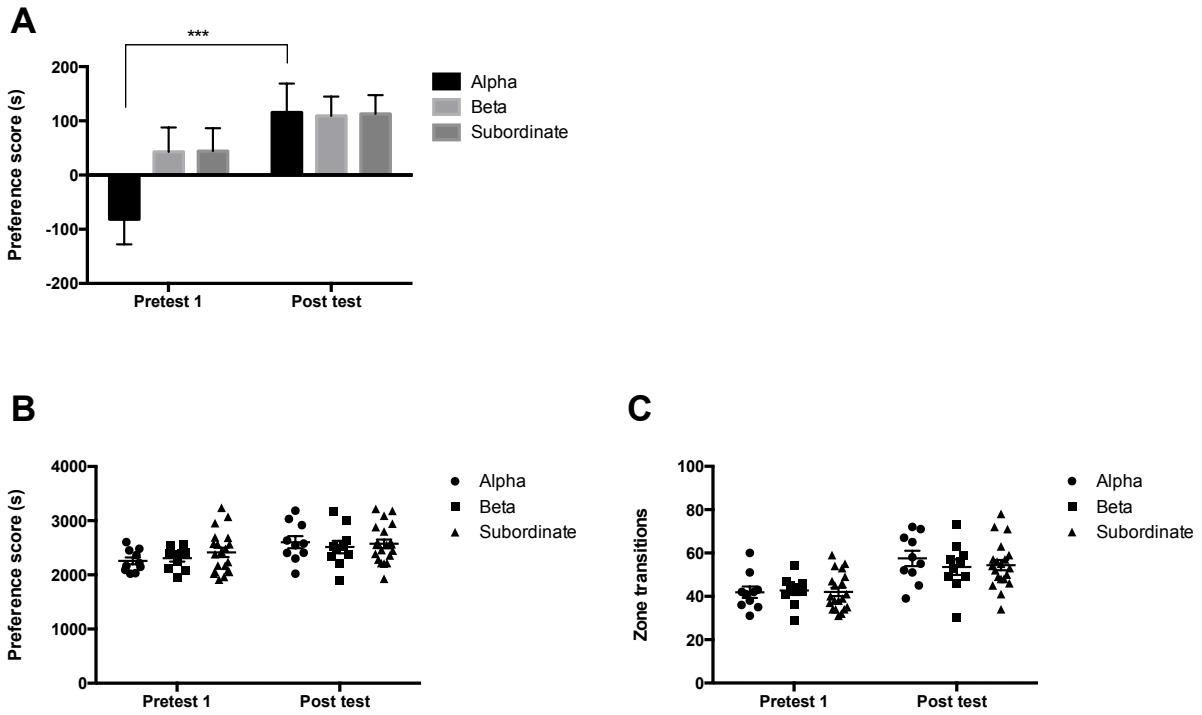
To investigate the impact of social hierarchy within the cage mice were ranked for their dominance using the dominance tube test, then animals underwent morphine CPP. We found that social status within the cage had no significant effect on the acquisition of morphine-CPP (figure B.2). One-way ANOVA with repeated measures revealed a significant effect of test ($F_{(1,37)}$=15.05 , p<0.001, n=10-20/treatment group) but not of treatment ($F_{(2,27)}$=0.25, p0.781, n=10-20/treatment group). Post hoc pairwise comparisons found no difference within repeated factor levels and only significant CPP was present in the Alpha mouse group (pre-test v post-test: p=0.003, n=10). We also saw no effect on the number of crosses (zone transitions) made during the post-test (Alpha v Beta: p0.731; Subordinate v Alpha: p0.752; Subordinate v Beta: p0.932, n=10-20/treatment group) nor the distance moved (Alpha v Beta: p0.947; Subordinate v Alpha: p0.946; Subordinate v Beta: p 0.992; n=10-20/treatment).

We found no significant effect of social hierarchy on reinstatement to morphine CPP (figure B.3). One-way ANOVA with repeated measures revealed a significant effect of test ($F_{(3,111)}$=7.89 , p<0.001, n=10-20/treatment group) but not of treatment ($F_{(4,24)}$=0.82, p0.524, n=10-20/treatment group). *Post hoc* pairwise comparisons found no difference within repeated factor levels (Reinstatement Alpha v Beta: p0.925; Beta v Subordinate: p0.724; Alpha v Subordinate: 0.808, n=10-20/treatment group.) We also saw no effect on the number of crosses (zone transitions) made during reinstatement (Alpha v Subordinate: p0.303; Alpha v Beta: p0.676; Subordinate v Beta: p0.133) nor the distance moved (Alpha v Beta: p0.688; Subordinate v Alpha: p0.140; Subordinate v Beta: p0.305).



Appendix B- 2 The effect of social hierarchy on the acquisition of morphine-CPP.

To investigate the impact of social hierarchy within the cage mice were ranked for their dominance using the dominance tube test, then animals underwent morphine CPP. One-way ANOVA with repeated measures revealed a significant effect of test ($F_{(1,37)}$=15.05 , p<0.001, n=10-20/treatment group) but not of treatment ($F_{(2,27)}$=0.25, p0.781, n=10-20/treatment group). *Post hoc* pairwise comparisons found no difference within repeated factor levels and only significant CPP was present in the Alpha mouse group (pre-test v post-test: p=0.003, n=10). We also saw no effect on the no. of crosses (zone transitions) made during the post-test (Alpha v Beta: p0.731; Subordinate v Alpha:p0.752; Subordinate v Beta: p0.932, n=10-20/treatment group) nor the distance moved (Alpha v Beta: p0.947; Subordinate v Alpha: p0.946; Subordinate v Beta: p 0.992; n=10-20/treatment).



Appendix B- 3 The effect of social hierarchy on the reinstatement of morphine-CPP.

To investigate the impact of social hierarchy within the cage mice were ranked for their dominance using the dominance tube test, then animals underwent reinstatement. One-way ANOVA with repeated measures revealed a significant effect of test ($F_{(3,111)}$=7.89 , p<0.001, n=10-20/treatment group) but not of treatment ($F_{(4,24)}$=0.82, p0.524, n=10-20/treatment group). Po*st hoc* found no difference within repeated factor levels (Reinstatement Alpha v Beta: p0.925; Beta v Subordinate: p0.724; Alpha v Subordinate: 0.808, n=10-20/treatment group.) We also saw no effect on the no. of crosses (zone transitions) made during reinstatement

APPENDIX C - AUTORADIOGRAPHY STATISTICS

C.1 [^{3}H]AMPA binding statistics

Table Analyzed	vHPC				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	5.493	0.1966	ns	No	
pretreatment	11.17	0.0722	ns	No	
treatment	25.22	0.0104	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2618	1	2618	F (1, 17) = 1.807	P = 0.1966
pretreatment	5326	1	5326	F (1, 17) = 3.675	P = 0.0722
treatment	12022	1	12022	F (1, 17) = 8.295	P = 0.0104
Residual	24637	17	1449		

Table Analyzed	dHPC				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.555	0.4232	ns	No	
pretreatment	24.43	0.0508	ns	No	
treatment	2.794	0.4027	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	581.5	1	581.5	F (1, 18) = 0.6715	P = 0.4232
pretreatment	5562	1	5562	F (1, 18) = 6.423	P = 0.0208
treatment	636.1	1	636.1	F (1, 18) = 0.7345	P = 0.4027
Residual	15588	18	866.0		

Table Analyzed	PrL				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	11.73	0.1064	ns	No	
pretreatment	12.26	0.0993	ns	No	
treatment	4.122	0.3269	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3177	1	3177	F (1, 18) = 2.889	P = 0.1064
pretreatment	3323	1	3323	F (1, 18) = 3.021	P = 0.0993
treatment	1117	1	1117	F (1, 18) = 1.016	P = 0.3269
Residual	19796	18	1100		

Table Analyzed	CA1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.04939	0.8496	ns	No	
pretreatment	16.46	0.0027	**	Yes	
treatment	50.59	< 0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	46.98	1	46.98	F (1, 17) = 0.03708	P = 0.8496
pretreatment	15654	1	15654	F (1, 17) = 12.36	P = 0.0027
treatment	48117	1	48117	F (1, 17) = 37.98	P < 0.0001
Residual	21537	17	1267		

Table Analyzed	CA2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	11.56	0.0456	*	Yes	
pretreatment	13.71	0.0311	*	Yes	
treatment	25.36	0.0053	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10926	1	10926	F (1, 17) = 4.654	P = 0.0456
pretreatment	12966	1	12966	F (1, 17) = 5.523	P = 0.0311
treatment	23975	1	23975	F (1, 17) = 10.21	P = 0.0053
Residual	39908	17	2348		

Table Analyzed	CA1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.04939	0.8496	ns	No	
pretreatment	16.46	0.0027	**	Yes	
treatment	50.59	< 0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	46.98	1	46.98	F (1, 17) = 0.03708	P = 0.8496
pretreatment	15654	1	15654	F (1, 17) = 12.36	P = 0.0027
treatment	48117	1	48117	F (1, 17) = 37.98	P < 0.0001
Residual	21537	17	1267		

Table Analyzed	CA3				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.753	0.5571	ns	No	
pretreatment	2.136	0.5173	ns	No	
treatment	10.99	0.1520	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	969.6	1	969.6	F (1, 17) = 0.3588	P = 0.5571
pretreatment	1182	1	1182	F (1, 17) = 0.4373	P = 0.5173
treatment	6079	1	6079	F (1, 17) = 2.249	P = 0.1520
Residual	45942	17	2702		

Table Analyzed	IL				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.964	0.4470	ns	No	
pretreatment	0.05247	0.9188	ns	No	
treatment	8.739	0.1986	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	985.5	1	985.5	F (1, 18) = 0.6043	P = 0.4470
pretreatment	17.45	1	17.45	F (1, 18) = 0.01070	P = 0.9188
treatment	2906	1	2906	F (1, 18) = 1.782	P = 0.1986
Residual	29356	18	1631		

Table Analyzed	M1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	5.501	0.3071	ns	No	
pretreatment	3.548	0.4097	ns	No	
treatment	0.9485	0.6677	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1030	1	1030	F (1, 18) = 1.105	P = 0.3071
pretreatment	664.5	1	664.5	F (1, 18) = 0.7125	P = 0.4097
treatment	177.7	1	177.7	F (1, 18) = 0.1905	P = 0.6677
Residual	16789	18	932.7		

Table Analyzed	M2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	8.775	0.1885	ns	No	
pretreatment	5.571	0.2905	ns	No	
treatment	0.6958	0.7048	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1222	1	1222	F (1, 18) = 1.868	P = 0.1885
pretreatment	775.5	1	775.5	F (1, 18) = 1.186	P = 0.2905
treatment	96.86	1	96.86	F (1, 18) = 0.1481	P = 0.7048
Residual	11770	18	653.9		

Table Analyzed	CgCx				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	10.17	0.1549	ns	No	
pretreatment	6.550	0.2489	ns	No	
treatment	0.5045	0.7447	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2578	1	2578	F (1, 18) = 2.205	P = 0.1549
pretreatment	1660	1	1660	F (1, 18) = 1.420	P = 0.2489
treatment	127.8	1	127.8	F (1, 18) = 0.1093	P = 0.7447
Residual	21045	18	1169		

Table Analyzed	CPu				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	5.359	0.2905	ns	No	
pretreatment	12.52	0.1132	ns	No	
treatment	0.3206	0.7930	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	277.8	1	277.8	F (1, 18) = 1.186	P = 0.2905
pretreatment	649.2	1	649.2	F (1, 18) = 2.772	P = 0.1132
treatment	16.62	1	16.62	F (1, 18) = 0.07095	P = 0.7930
Residual	4216	18	234.2		

Table Analyzed	ACc				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.378	0.5002	ns	No	
pretreatment	3.170	0.4373	ns	No	
treatment	3.398	0.4215	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	731.6	1	731.6	F (1, 18) = 0.4734	P = 0.5002
pretreatment	975.4	1	975.4	F (1, 18) = 0.6312	P = 0.4373
treatment	1046	1	1046	F (1, 18) = 0.6766	P = 0.4215
Residual	27817	18	1545		

Table Analyzed	ACs				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	6.934	0.2617	ns	No	
pretreatment	0.1075	0.8869	ns	No	
treatment	0.01905	0.9522	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2368	1	2368	F (1, 18) = 1.343	P = 0.2617
pretreatment	36.70	1	36.70	F (1, 18) = 0.02081	P = 0.8869
treatment	6.505	1	6.505	F (1, 18) = 0.003689	P = 0.9522
Residual	31741	18	1763		

Table Analyzed AMG	AMG				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	4.386	0.3302	ns	No	
pretreatment	15.17	0.0791	ns	No	
treatment	0.8346	0.6676	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	547.6	1	547.6	F (1, 18) = 1.001	P = 0.3302
pretreatment	1894	1	1894	F (1, 18) = 3.464	P = 0.0791
treatment	104.2	1	104.2	F (1, 18) = 0.1906	P = 0.6676
Residual	9843	18	546.8		

Table Analyzed	CeA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.649	0.4556	ns	No	
pretreatment	6.604	0.2441	ns	No	
treatment	7.364	0.2197	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	573.8	1	573.8	F (1, 18) = 0.5815	P = 0.4556
pretreatment	1431	1	1431	F (1, 18) = 1.450	P = 0.2441
treatment	1595	1	1595	F (1, 18) = 1.617	P = 0.2197
Residual	17761	18	986.7		

Table Analyzed BMA	BMA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	14.65	0.0659	ns	No	
pretreatment	9.907	0.1248	ns	No	
treatment	5.211	0.2582	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3226	1	3226	F (1, 18) = 3.833	P = 0.0659
pretreatment	2182	1	2182	F (1, 18) = 2.592	P = 0.1248
treatment	1147	1	1147	F (1, 18) = 1.363	P = 0.2582
Residual	15148	18	841.5		

Table Analyzed	VTA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	13.74	0.0914	ns	No	
pretreatment	1.295	0.5900	ns	No	
treatment	10.84	0.1305	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1029	1	1029	F (1, 17) = 3.201	P = 0.0914
pretreatment	96.95	1	96.95	F (1, 17) = 0.3016	P = 0.5900
treatment	811.7	1	811.7	F (1, 17) = 2.525	P = 0.1305
Residual	5465	17	321.5		

Table Analyzed	AuCx				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	7.983	0.1783	ns	No	
pretreatment	18.76	0.0460	*	Yes	
treatment	2.598	0.4342	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1396	1	1396	F (1, 17) = 1.972	P = 0.1783
pretreatment	3281	1	3281	F (1, 17) = 4.633	P = 0.0460
treatment	454.4	1	454.4	F (1, 17) = 0.6415	P = 0.4342
Residual	12041	17	708.3		

Table Analyzed	ViCx				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.6574	0.6657	ns	No	
pretreatment	9.410	0.1146	ns	No	
treatment	26.30	0.0128	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	161.6	1	161.6	F (1, 17) = 0.1933	P = 0.6657
pretreatment	2313	1	2313	F (1, 17) = 2.767	P = 0.1146
treatment	6465	1	6465	F (1, 17) = 7.732	P = 0.0128
Residual	14215	17	836.2		

Table Analyzed	dCA1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.5475	0.7565	ns	No	
pretreatment	0.06517	0.9147	ns	No	
treatment	0.002255	0.9841	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	806.7	1	806.7	F (1, 18) = 0.09917	P = 0.7565
pretreatment	96.02	1	96.02	F (1, 18) = 0.01180	P = 0.9147
treatment	3.323	1	3.323	F (1, 18) = 0.0004085	P = 0.9841
Residual	146428	18	8135		

Table Analyzed	dCA2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.03143	0.9387	ns	No	
pretreatment	0.6880	0.7196	ns	No	
treatment	5.723	0.3069	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	36.99	1	36.99	F (1, 18) = 0.006074	P = 0.9387
pretreatment	809.8	1	809.8	F (1, 18) = 0.1330	P = 0.7196
treatment	6736	1	6736	F (1, 18) = 1.106	P = 0.3069
Residual	109634	18	6091		

Table Analyzed	dCA3				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	6.324	0.2766	ns	No	
pretreatment	0.5492	0.7447	ns	No	
treatment	2.904	0.4569	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3488	1	3488	F (1, 18) = 1.259	P = 0.2766
pretreatment	302.9	1	302.9	F (1, 18) = 0.1093	P = 0.7447
treatment	1602	1	1602	F (1, 18) = 0.5782	P = 0.4569
Residual	49873	18	2771		

C.2 **[3H]MK801 binding statistics**

Table Analyzed	Prl				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.1638	0.8562	ns	No	
Pre-treatment	0.08928	0.8936	ns	No	
Treatment	2.306	0.4989	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	276.8	1	276.8	F (1, 20) = 0.03370	P = 0.8562
Pre-treatment	150.8	1	150.8	F (1, 20) = 0.01837	P = 0.8936
Treatment	3895	1	3895	F (1, 20) = 0.4743	P = 0.4989
Residual	164246	20	8212		

Table Analyzed	IL				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.1489	0.8621	ns	No	
Pre-treatment	0.02897	0.9389	ns	No	
Treatment	3.685	0.3917	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	238.2	1	238.2	F (1, 20) = 0.03098	P = 0.8621
Pre-treatment	46.34	1	46.34	F (1, 20) = 0.006027	P = 0.9389
Treatment	5894	1	5894	F (1, 20) = 0.7666	P = 0.3917
Residual	153782	20	7689		

Table Analyzed	M1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.4094	0.7624	ns	No	
Pre-treatment	2.211	0.4845	ns	No	
Treatment	8.441	0.1793	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	963.3	1	963.3	F (1, 20) = 0.09395	P = 0.7624
Pre-treatment	5202	1	5202	F (1, 20) = 0.5073	P = 0.4845
Treatment	19860	1	19860	F (1, 20) = 1.937	P = 0.1793
Residual	205066	20	10253		

Table Analyzed	M2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.01618	0.9524	ns	No	
Pre-treatment	2.802	0.4360	ns	No	
Treatment	6.734	0.2321	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	36.44	1	36.44	F (1, 20) = 0.003648	P = 0.9524
Pre-treatment	6310	1	6310	F (1, 20) = 0.6318	P = 0.4360
Treatment	15167	1	15167	F (1, 20) = 1.519	P = 0.2321
Residual	199753	20	9988		

Table Analyzed	CgCx				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.1341	0.8658	ns	No	
Pre-treatment	0.7565	0.6887	ns	No	
Treatment	6.598	0.2439	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	174.0	1	174.0	F (1, 20) = 0.02931	P = 0.8658
Pre-treatment	981.4	1	981.4	F (1, 20) = 0.1653	P = 0.6887
Treatment	8559	1	8559	F (1, 20) = 1.442	P = 0.2439
Residual	118749	20	5937		

Table Analyzed	CPu				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	7.635	0.2054	ns	No	
Pre-treatment	1.263	0.6004	ns	No	
Treatment	2.459	0.4663	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3524	1	3524	F (1, 20) = 1.713	P = 0.2054
Pre-treatment	583.1	1	583.1	F (1, 20) = 0.2834	P = 0.6004
Treatment	1135	1	1135	F (1, 20) = 0.5516	P = 0.4663
Residual	41150	20	2058		

Table Analyzed	AccC				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.1051	0.8815	ns	No	
Pre-treatment	0.5687	0.7291	ns	No	
Treatment	7.593	0.2141	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	64.36	1	64.36	F (1, 20) = 0.02278	P = 0.8815
Pre-treatment	348.4	1	348.4	F (1, 20) = 0.1233	P = 0.7291
Treatment	4651	1	4651	F (1, 20) = 1.647	P = 0.2141
Residual	56491	20	2825		

Table Analyzed	AccS				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.2968	0.7997	ns	No	
Pre-treatment	0.9174	0.6561	ns	No	
Treatment	7.835	0.2014	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	316.8	1	316.8	F (1, 20) = 0.06612	P = 0.7997
Pre-treatment	979.1	1	979.1	F (1, 20) = 0.2044	P = 0.6561
Treatment	8362	1	8362	F (1, 20) = 1.745	P = 0.2014
Residual	95815	20	4791		

Table Analyzed	dHPC				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.006441	0.9707	ns	No	
Pre-treatment	8.368	0.1951	ns	No	
Treatment	2.184	0.5009	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	12.27	1	12.27	F (1, 19) = 0.001388	P = 0.9707
Pre-treatment	15938	1	15938	F (1, 19) = 1.803	P = 0.1951
Treatment	4160	1	4160	F (1, 19) = 0.4708	P = 0.5009
Residual	167906	19	8837		

Table Analyzed	AMG				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.770	0.4416	ns	No	
Pre-treatment	2.071	0.5049	ns	No	
Treatment	8.162	0.1931	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2677	1	2677	F (1, 19) = 0.6178	P = 0.4416
Pre-treatment	2002	1	2002	F (1, 19) = 0.4621	P = 0.5049
Treatment	7891	1	7891	F (1, 19) = 1.821	P = 0.1931
Residual	82341	19	4334		

Table Analyzed	CeA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	8.246	0.1935	ns	No	
Pre-treatment	0.6443	0.7101	ns	No	
Treatment	8.342	0.1910	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	8199	1	8199	F (1, 18) = 1.825	P = 0.1935
Pre-treatment	640.7	1	640.7	F (1, 18) = 0.1426	P = 0.7101
Treatment	8295	1	8295	F (1, 18) = 1.846	P = 0.1910
Residual	80867	18	4493		

Table Analyzed	BLA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.617	0.4786	ns	No	
Pre-treatment	0.4018	0.7801	ns	No	
Treatment	1.366	0.6075	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2312	1	2312	F (1, 19) = 0.5225	P = 0.4786
Pre-treatment	355.0	1	355.0	F (1, 19) = 0.08023	P = 0.7801
Treatment	1207	1	1207	F (1, 19) = 0.2727	P = 0.6075
Residual	84068	19	4425		

Table Analyzed	BMA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.03847	0.9311	ns	No	
Pre-treatment	0.1061	0.8859	ns	No	
Treatment	4.236	0.3697	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	43.86	1	43.86	F (1, 19) = 0.007667	P = 0.9311
Pre-treatment	120.9	1	120.9	F (1, 19) = 0.02114	P = 0.8859
Treatment	4830	1	4830	F (1, 19) = 0.8443	P = 0.3697
Residual	108695	19	5721		

Table Analyzed	vHPC				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.277	0.5896	ns	No	
Pre-treatment	3.256	0.3918	ns	No	
Treatment	13.33	0.0923	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2516	1	2516	F (1, 19) = 0.3011	P = 0.5896
Pre-treatment	6417	1	6417	F (1, 19) = 0.7678	P = 0.3918
Treatment	26267	1	26267	F (1, 19) = 3.143	P = 0.0923
Residual	158799	19	8358		

Table Analyzed	CA1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.9938	0.6185	ns	No	
Pre-treatment	7.022	0.1943	ns	No	
Treatment	15.65	0.0589	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3360	1	3360	F (1, 19) = 0.2563	P = 0.6185
Pre-treatment	23742	1	23742	F (1, 19) = 1.811	P = 0.1943
Treatment	52926	1	52926	F (1, 19) = 4.037	P = 0.0589
Residual	249125	19	13112		

Table Analyzed	CA2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.1946	0.8293	ns	No	
Pre-treatment	5.484	0.2603	ns	No	
Treatment	14.06	0.0787	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	614.5	1	614.5	F (1, 19) = 0.04777	P = 0.8293
Pre-treatment	17320	1	17320	F (1, 19) = 1.346	P = 0.2603
Treatment	44420	1	44420	F (1, 19) = 3.453	P = 0.0787
Residual	244449	19	12866		

Table Analyzed	CA3				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.202	0.5080	ns	No	
Pre-treatment	1.545	0.5786	ns	No	
Treatment	3.952	0.3774	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	5255	1	5255	F (1, 19) = 0.4552	P = 0.5080
Pre-treatment	3687	1	3687	F (1, 19) = 0.3194	P = 0.5786
Treatment	9431	1	9431	F (1, 19) = 0.8170	P = 0.3774
Residual	219343	19	11544		

Table Analyzed	VTA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.708	0.4741	ns	No	
Pre-treatment	0.3552	0.7942	ns	No	
Treatment	0.2503	0.8266	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1017	1	1017	F (1, 19) = 0.5334	P = 0.4741
Pre-treatment	133.4	1	133.4	F (1, 19) = 0.06996	P = 0.7942
Treatment	94.00	1	94.00	F (1, 19) = 0.04932	P = 0.8266
Residual	36217	19	1906		

Table Analyzed	AuCX				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.002854	0.9807	ns	No	
Pre-treatment	5.643	0.2893	ns	No	
Treatment	2.904	0.4438	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2.355	1	2.355	F (1, 19) = 0.0006010	P = 0.9807
Pre-treatment	4656	1	4656	F (1, 19) = 1.188	P = 0.2893
Treatment	2396	1	2396	F (1, 19) = 0.6116	P = 0.4438
Residual	74447	19	3918		

Table Analyzed	ViCX				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.6850	0.6953	ns	No	
Pre-treatment	2.920	0.4220	ns	No	
Treatment	15.73	0.0726	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	709.3	1	709.3	F (1, 18) = 0.1584	P = 0.6953
Pre-treatment	3024	1	3024	F (1, 18) = 0.6751	P = 0.4220
Treatment	16286	1	16286	F (1, 18) = 3.636	P = 0.0726
Residual	80619	18	4479		

Table Analyzed	dCA1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.723	0.4431	ns	No	
Pre-treatment	6.436	0.2436	ns	No	
Treatment	9.572	0.1587	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10873	1	10873	F (1, 18) = 0.6150	P = 0.4431
Pre-treatment	25695	1	25695	F (1, 18) = 1.453	P = 0.2436
Treatment	38216	1	38216	F (1, 18) = 2.162	P = 0.1587
Residual	318203	18	17678		

Table Analyzed	dCA2				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	3.604	0.3779	ns	No	
Pre-treatment	6.039	0.2571	ns	No	
Treatment	9.475	0.1599	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	13762	1	13762	F (1, 18) = 0.8173	P = 0.3779
Pre-treatment	23062	1	23062	F (1, 18) = 1.370	P = 0.2571
Treatment	36184	1	36184	F (1, 18) = 2.149	P = 0.1599
Residual	303096	18	16839		

Table Analyzed	dCA3				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	3.504	0.3863	ns	No	
Pre-treatment	0.4404	0.7565	ns	No	
Treatment	15.45	0.0787	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10552	1	10552	F (1, 18) = 0.7884	P = 0.3863
Pre-treatment	1326	1	1326	F (1, 18) = 0.09910	P = 0.7565
Treatment	46517	1	46517	F (1, 18) = 3.476	P = 0.0787
Residual	240894	18	13383		

Bibliography

Abdulla F a, Bradbury E, Calaminici MR, Lippiello PM, Wonnacott S, Gray J a, Sinden JD. **1996**. Relationship between up-regulation of nicotine binding sites in rat brain and delayed cognitive enhancement observed after chronic or acute nicotinic receptor stimulation. *Psychopharmacology (Berl).* **124**: 323–331.

Acker BA, Jacobsen EJ, Rogers BN, Wishka DG, Reitz SC, Piotrowski DW, Myers JK, Wolfe ML, Groppi VE, Thornburgh BA, Tinholt PM, Walters RR, Olson BA, Fitzgerald L, Staton BA, Raub TJ, Krause M, Li KS, Hoffmann WE, Hajos M, Hurst RS, Walker DP. **2008**. Discovery of N-[(3R,5R)-1-azabicyclo[3.2.1]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide as an agonist of the alpha7 nicotinic acetylcholine receptor: in vitro and in vivo activity. *Bioorg. Med. Chem. Lett.* **18**: 3611–5.

Action on Smoking and Health. **2015**. Smoking statistics. *Can. Fam. Physician* **27**: 402.

Addy N a, Nakijama A, Levin ED. **2003**. Nicotinic mechanisms of memory: effects of acute local DHbetaE and MLA infusions in the basolateral amygdala. *Brain Res. Cogn. Brain Res.* **16**: 51–7.

Agmo a, Galvan a, Talamantes B. **1995**. Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. *Pharmacol. Biochem. Behav.* **52**: 403–14.

Aguilar M a, Rodríguez-Arias M, Miñarro J. **2009**. Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res. Rev.* **59**: 253–77.

Ahmadi S, Zarrindast MR, Haeri-Rohani A, Rezayof A, Nouri M. **2007**. Nicotine improves morphine-induced impairment of memory: possible involvement of N-methyl-D-aspartate receptors in the nucleus accumbens. *Dev. Neurobiol.* **67**: 1118–27.

Ahmed SH, Koob GF. **2005**. Transition to drug addiction: a negative reinforcement model based on an allostatic decrease in reward function. *Psychopharmacology (Berl).* **180**: 473–90.

Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. **2009**. Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol. Rev.* **89**: 73–120.

Alkondon M, Albuquerque EX. **2001**. Nicotinic acetylcholine receptor a7 and a4b2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *J. Neurophysiol.* **86**: 3043–3055.

Alkondon M, Pereira EF, Albuquerque EX. **1998**. Alpha-Bungarotoxin- and Methyllycaconitine-Sensitive Nicotinic Receptors Mediate Fast Synaptic

Transmission in Interneurons of Rat Hippocampal Slices. *Brain Res.* **810**: 257–263.

Alkondon M, Pereira EF, Barbosa CT, Albuquerque EX. **1997**. Neuronal nicotinic acetylcholine receptor activation modulates gamma-aminobutyric acid release from CA1 neurons of rat hippocampal slices. *J. Pharmacol. Exp. Ther.* **283**: 1396–1411.

Alkondon M, Rocha ES, Maelicke A, Albuquerque EX. **1996**. Diversity of nicotinic acetylcholine receptors in rat brain. V. alpha-Bungarotoxin-sensitive nicotinic receptors in olfactory bulb neurons and presynaptic modulation of glutamate release. *J. Pharmacol. Exp. Ther.* **278**: 1460–71.

Amaral DG, Kurz J. **1985**. An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. *J. Comp. Neurol.* **240**: 37–59.

Amaral DG, Witter MP. **1989**. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* **31**: 571–91.

Amaral, Witter. **1995**. Hippocampal formation. In: Paxinos G, editor. The rat nervous system, 2nd ed. *Los Angeles, CA 1995. p. 443-493*: p443–493.

Andrásfalvy BK, Magee JC. **2004**. Changes in AMPA receptor currents following LTP induction on rat CA1 pyramidal neurones. *J. Physiol.* **559**: 543–554.

Andriambeloson E, Huyard B, Poiraud E, Wagner S. **2014**. Methyllycaconitine- and scopolamine-induced cognitive dysfunction: differential reversal effect by cognition-enhancing drugs. *Pharmacol. Res. Perspect.* **2**: n/a–n/a.

Aosaki T, Graybiel AM, Kimura M. **1994**. Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. *Science* **265**: 412–5.

Arai A, Larson J, Lynch G. **1990**. Anoxia reveals a vulnerable period in the development of long-term potentiation. *Brain Res.* **511**: 353–357.

Arendash GW, Sengstock GJ, Sanberg PR, Kem WR. **1995**. Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. *Brain Res.* **674**: 252–9.

Aznavour N, Mechawar N, Descarries L. **2002**. Comparative analysis of cholinergic innervation in the dorsal hippocampus of adult mouse and rat: A quantitative immunocytochemical study. *Hippocampus* **12**: 206–217.

Bachtell RK, Whisler K, Karanian D, Self DW. **2005**. Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology (Berl).* **183**: 41–53.

Bailey A, Metaxas A, Al-Hasani R, Keyworth HL, Forster DM, Kitchen I. **2010**. Mouse strain differences in locomotor, sensitisation and rewarding effect of heroin; association with alterations in MOP-r activation and dopamine transporter binding. *Eur. J. Neurosci.* **31**: 742–53.

Baler RD, Volkow ND. **2006**. Drug addiction: the neurobiology of disrupted self-control. *Trends Mol. Med.* **12**: 559–566.

Banerjee S, Punzi JS, Kreilick K, Abood LG. **1990**. [3H]mecamylamine binding to rat brain membranes. Studies with mecamylamine and nicotine analogues. *Biochem. Pharmacol.* **40**: 2105–10.

Bannerman DM, Rawlins JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, Zhang WN, Pothuizen HHJ, Feldon J. **2004**. Regional dissociations within the hippocampus - Memory and anxiety. *Neurosci. Biobehav. Rev.* **28**: 273–283.

Bardo MT, Bevins R a. **2000**. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl).* **153**: 31–43.

Bardo MT, Neisewander JL. **1986**. Single-trial conditioned place preference using intravenous morphine. *Pharmacol. Biochem. Behav.* **25**: 1101–5.

Bardo MT, Rowlett JK, Harris MJ. **1995**. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. *Neurosci. Biobehav. Rev.* **19**: 39–51.

Barrionuevo G, Schottler F, Lynch G. **1980**. The effects of repetitive low frequency stimulation on control and 'potentiated' synaptic responses in the hippocampus. *Life Sci.* **27**: 2385–91.

Barron AB, Søvik E, Cornish JL. **2010**. The roles of dopamine and related compounds in reward-seeking behavior across animal phyla. *Front. Behav. Neurosci.* **4**: 163.

BBC. **2004**. BBC NEWS | World | Americas | Boozy bear plunders campers' beer.

Belluzzi JD, Lee AG, Oliff HS, Leslie FM. **2004**. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology (Berl).* **174**: 389–95.

Benke TA, Lüthi A, Isaac JT, Collingridge GL. **1998**. Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393**: 793–7.

Benowitz NL, Hatsukami D. **1998**. Gender differences in the pharmacology of nicotine addiction. *Addict. Biol.* **3**: 383–404.

Bertrand D, Ballivet M, Rungger D. **1990**. Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in Xenopus oocytes. *Proc. Natl. Acad. Sci. U. S. A.* **87**: 1993–7.

Biala G, Budzynska B. **2006**. Reinstatement of nicotine-conditioned place preference by drug priming: effects of calcium channel antagonists. *Eur. J. Pharmacol.* **537**: 85–93.

Biala G, Staniak N. **2010**. Varenicline and mecamylamine attenuate locomotor sensitization and cross-sensitization induced by nicotine and morphine in mice. *Pharmacol. Biochem. Behav.* **96**: 141–7.

Biala G, Staniak N, Budzynska B. **2010**. Effects of varenicline and mecamylamine on the acquisition, expression, and reinstatement of nicotine-conditioned place preference by drug priming in rats. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **381**: 361–70.

Biala G, Weglinska B. **2004**. Calcium channel antagonists attenuate cross-sensitization to the rewarding and/or locomotor effects of nicotine, morphine and MK-801. *J. Pharm. Pharmacol.* **56**: 1021–8.

Billa SK, Liu J, Bjorklund NL, Sinha N, Fu Y, Shinnick-gallagher P, Moro JA. **2010**. Increased Insertion of Glutamate Receptor 2-Lacking ‿ -Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid (AMPA) Receptors at Hippocampal Synapses upon Repeated Morphine Administration. **77**: 874–883.

Billa SK, Sinha N, Rudrabhatla SR, Morón J a. **2009**. Extinction of morphine-dependent conditioned behavior is associated with increased phosphorylation of the GluR1 subunit of AMPA receptors at hippocampal synapses. *Eur. J. Neurosci.* **29**: 55–64.

Bindra D. **1974**. A motivational view of learning, performance, and behavior modification. *Psychol. Rev.* **81**: 199–213.

Bitner RS, Bunnelle WH, Anderson DJ, Briggs CA, Buccafusco J, Curzon P, Decker MW, Frost JM, Gronlien JH, Gubbins E, Li J, Malysz J, Markosyan S, Marsh K, Meyer MD, Nikkel AL, Radek RJ, Robb HM, Timmermann D, Sullivan JP, Gopalakrishnan M. **2007**. Broad-spectrum efficacy across cognitive domains by alpha7 nicotinic acetylcholine receptor agonism correlates with activation of ERK1/2 and CREB phosphorylation pathways. *J. Neurosci.* **27**: 10578–87.

Biton B, Bergis OE, Galli F, Nedelec A, Lochead AW, Jegham S, Godet D, Lanneau C, Santamaria R, Chesney F, Léonardon J, Granger P, Debono MW, Bohme GA, Sgard F, Besnard F, Graham D, Coste A, Oblin A, Curet O, Vigé X, Voltz C, Rouquier L, Souilhac J, Santucci V, Gueudet C, Françon D, Steinberg R, Griebel G, Oury-Donat F, George P, Avenet P, Scatton B. **2007**. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (1) binding and functional profile. *Neuropsychopharmacology* **32**: 1–16.

Bittar P, Muller D. **1993**. Time-dependent reversal of long-term potentiation by brief cooling shocks in rat hippocampal slices. *Brain Res.* **620**: 181–188.

Blackstone CD, Moss SJ, Martin LJ, Levey AI, Price DL, Huganir RL. **1992**. Biochemical characterization and localization of a non-N-methyl-D-aspartate glutamate receptor in rat brain. *J. Neurochem.* **58**: 1118–26.

Blake MG, Krawczyk MC, Baratti CM, Boccia MM. **2014**. Neuropharmacology of memory consolidation and reconsolidation: Insights on central cholinergic mechanisms. *J. Physiol.*

Bland BH, Oddie SD. **2001**. Theta band oscillation and synchrony in the hippocampal formation and associated structures: the case for its role in sensorimotor integration. *Behav. Brain Res.* **127**: 119–36.

Bliss T, Collingridge GL. **1993**. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**: 31–39.

Bliss T V, Lomo T. **1973**. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**: 331–56.

Bloem B, Poorthuis RB, Mansvelder HD. **2014**. Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. *Front. Neural Circuits* **8**: 17.

Boccia MM, Blake MG, Krawczyk MC, Baratti CM. **2010**. Hippocampal α7 nicotinic receptors modulate memory reconsolidation of an inhibitory avoidance task in mice. *Neuroscience* **171**: 531–43.

Boess FG, De Vry J, Erb C, Flessner T, Hendrix M, Luithle J, Methfessel C, Riedl B, Schnizler K, van der Staay F-J, van Kampen M, Wiese WB, Koenig G. **2007**. The novel alpha7 nicotinic acetylcholine receptor agonist N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide improves working and recognition memory in rodents. *J. Pharmacol. Exp. Ther.* **321**: 716–25.

Bolles RC. **1972**. Reinforcement, expectancy, and learning. *Psychol. Rev.* **79**: 394–409.

Borges K, Dingledine R. **1998**. AMPA receptors: molecular and functional diversity. *Prog. Brain Res.* **116**: 153–70.

Boudreau AC, Milovanovic M, Conrad KL, Nelson C, Carrie R, Wolf ME. **2012**. A protein crosslinking assay for measuring cell surface expression of gluatmate receptor subunits in the rodent brain after in vivo treatments. *Curr. Protoc. Neurosci.*: 1–22.

Boudreau AC, Wolf ME. **2005**. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J. Neurosci.* **25**: 9144–51.

Bozarth MA. **1987**. Assessing Drug Reinforcement, Chapter 14 (MA Bozarth, Ed.). New York: Springer-Verlag.

Brenhouse HC, Dumais K, Andersen SL. **2010**. Enhancing the salience of dullness: behavioral and pharmacological strategies to facilitate extinction of drug-cue associations in adolescent rats. *Neuroscience* **169**: 628–36.

Briggs CA, Anderson DJ, Brioni JD, Buccafusco JJ, Buckley MJ, Campbell JE, Decker MW, Donnelly-Roberts D, Elliott RL, Gopalakrishnan M, Holladay MW, Hui YH, Jackson WJ, Kim DJ, Marsh KC, O'Neill A, Prendergast MA, Ryther KB, Sullivan JP, Arneric SP. **1997**. Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo. *Pharmacol. Biochem. Behav.* **57**: 231–41.

Brioni JD, Decker MW, Sullivan JP, Arneric SP. **1997**. The pharmacology of (-)-nicotine and novel cholinergic channel modulators. *Adv. Pharmacol.* **37**: 153–214.

Brito GN, Brito LS. **1990**. Septohippocampal system and the prelimbic sector of frontal cortex: a neuropsychological battery analysis in the rat. *Behav. Brain Res.* **36**: 127–46.

Broide RS, Leslie FM. **1999**. The alpha7 nicotinic acetylcholine receptor in neuronal plasticity. *Mol. Neurobiol.* **20**: 1–16.

Buccafusco JJ, Terry A V, Decker MW, Gopalakrishnan M. **2007**. Profile of nicotinic acetylcholine receptor agonists ABT-594 and A-582941, with differential subtype selectivity, on delayed matching accuracy by young monkeys. *Biochem. Pharmacol.* **74**: 1202–11.

Budzyńska B, Biała G. **2011**. Effects of bupropion on the reinstatement of nicotine-induced conditioned place preference by drug priming in rats. *Pharmacol. Reports* **63**: 362–371.

Burton BG, Hok V, Save E, Poucet B. **2009**. Lesion of the ventral and intermediate hippocampus abolishes anticipatory activity in the medial prefrontal cortex of the rat. *Behav. Brain Res.* **199**: 222–34.

Burwell RD. **2000**. The parahippocampal region: corticocortical connectivity. *Ann. N. Y. Acad. Sci.* **911**: 25–42.

Caffino L, Frankowska M, Giannotti G, Miszkiel J, Sadakierska-Chudy A, Racagni G, Filip M, Fumagalli F. **2014**. Cocaine-induced glutamate receptor trafficking is abrogated by extinction training in the rat hippocampus. *Pharmacol. Reports* **66**: 198–204.

Caine SB, Thomsen M, Gabriel KI, Berkowitz JS, Gold LH, Koob GF, Tonegawa S, Zhang J, Xu M. **2007**. Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. *J. Neurosci.* **27**: 13140–50.

Carlezon W a, Duman RS, Nestler EJ. **2005**. The many faces of CREB. *Trends Neurosci.* **28**: 436–45.

Carlezon WA, Nestler EJ. **2002**. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci.* **25**: 610–5.

Cenquizca LA, Swanson LW. **2007**. Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Res. Rev.* **56**: 1–26.

Champtiaux N, Han Z-Y, Bessis A, Rossi FM, Zoli M, Marubio L, McIntosh JM, Changeux J-P. **2002**. Distribution and pharmacology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. *J. Neurosci.* **22**: 1208–1217.

Champtiaux N, Kalivas PW, Bardo MT. **2006**. Contribution of dihydro-beta-erythroidine sensitive nicotinic acetylcholine receptors in the ventral tegmental area to cocaine-induced behavioral sensitization in rats. *Behav. Brain Res.* **168**: 120–6.

Chandler JP, Crutcher KA. **1983**. The septohippocampal projection in the rat: An electron microscopic horseradish peroxidase study. *Neuroscience* **10**: 685–696.

Chang KT, Berg DK. **2001**. Voltage-gated channels block nicotinic regulation of CREB phosphorylation and gene expression in neurons. *Neuron* **32**: 855–65.

Changeux J, Devillers-Thiery A, Chemouilli P. **1984**. Acetylcholine receptor: an allosteric protein. *Science (80-.).* **225**: 1335–1345.

Changeux JP, Kasai M, Lee CY. **1970**. Use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 1241–7.

Chatterjee S, Steensland P, Simms J a, Holgate J, Coe JW, Hurst RS, Shaffer CL, Lowe J, Rollema H, Bartlett SE. **2011**. Partial agonists of the α3β4* neuronal nicotinic acetylcholine receptor reduce ethanol consumption and seeking in rats. *Neuropsychopharmacology* **36**: 603–15.

Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ, Johnson EC. **1997**. Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha 2 beta 2, h alpha 2 beta 4, h alpha 3 beta 2, h alpha 3 beta 4, h alpha 4 beta 2, h alpha 4 beta 4 and h alpha 7 expressed in Xenopus oocytes. *J. Pharmacol. Exp. Ther.* **280**: 346–56.

Cheng Q, Yakel JL. **2015**. The effect of α7 nicotinic receptor activation on glutamatergic transmission in the hippocampus. *Biochem. Pharmacol.*

Di Chiara G, Imperato A. **1988**. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U. S. A.* **85**: 5274–8.

Chilton M, Mastropaolo J, Rosse RB, Bellack AS, Deutsch SI. **2004**. Behavioral consequences of methyllycaconitine in mice: a model of alpha7 nicotinic acetylcholine receptor deficiency. *Life Sci.* **74**: 3133–9.

Choquet D, Triller A. **2003**. The role of receptor diffusion in the organization of the postsynaptic membrane. *Nat. Rev. Neurosci.* **4**: 251–65.

Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM. **1987**. Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. *Neuroscience* **22**: 425–39.

Clarke PB, Reuben M. **1996**. Release of [3H]-noradrenaline from rat hippocampal synaptosomes by nicotine: mediation by different nicotinic receptor subtypes from striatal [3H]-dopamine release. *Br. J. Pharmacol.* **117**: 595–606.

Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A. **1985**. Nicotinic binding in rat brain: autoradiographic comparison of [3H]acetylcholine, [3H]nicotine, and [125I]-alpha-bungarotoxin. *J. Neurosci.* **5**: 1307–15.

Cobb SR, Davies CH. **2005**. Cholinergic modulation of hippocampal cells and circuits. *J. Physiol.* **562**: 81–8.

Cole RL, Konradi C, Douglass J, Hyman SE. **1995**. Neuronal adaptation to amphetamine and dopamine: Molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* **14**: 813–823.

Corrigall WA, Coen KM, Adamson KL. **1994**. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res.* **653**: 278–84.

Corrigall WA, Franklin KB, Coen KM, Clarke PB. **1992**. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl).* **107**: 285–9.

Do Couto BR, Aguilar MA, Manzanedo C, Rodríguez-Arias M, Miñarro J. **2003**. Reinstatement of Morphine-Induced Conditioned Place Preference in Mice by Priming Injections. *Neural Plast.* **10**: 279–290.

Crespo J a, Sturm K, Saria A, Zernig G. **2006**. Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement. *J. Neurosci.* **26**: 6004–6010.

Crooks PA, Bardo MT, Dwoskin LP. **2014**. Nicotinic receptor antagonists as treatments for nicotine abuse. *Adv. Pharmacol.* **69**: 513–51.

Crutcher KA, Madison R, Davis JN. **1981**. A study of the rat septohippocampal pathway using anterograde transport of horseradish peroxidase. *Neuroscience* **6**: 1961–1973.

Dela Cruz AM, Herin D V, Grady JJ, Cunningham KA. **2009**. Novel approach to data analysis in cocaine-conditioned place preference. *Behav. Pharmacol.* **20**: 720–30.

Cruz FC, Marin MT, Planeta CS. **2008**. The reinstatement of amphetamine-induced place preference is long-lasting and related to decreased expression of AMPA receptors in the nucleus accumbens. *Neuroscience* **151**: 313–9.

Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, Salminen O, Tritto T, Butt CM, Allen WR, Stitzel JA, McIntosh JM, Boulter J, Collins AC, Heinemann SF. **2003**. The beta3 nicotinic receptor subunit: a component of alpha-conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. *J. Neurosci.* **23**: 11045–53.

Cull-Candy S, Kelly L, Farrant M. **2006**. Regulation of Ca2+-permeable AMPA receptors: synaptic plasticity and beyond. *Curr. Opin. Neurobiol.* **16**: 288–97.

Cunningham CL, Gremel CM, Groblewski PA. **2006**. Drug-induced conditioned place preference and aversion in mice. *Nat. Protoc.* **1**: 1662–70.

Cunningham CL, Zerizef CL. **2014**. Effects of combining tactile with visual and spatial cues in conditioned place preference. *Pharmacol. Biochem. Behav.* **124**: 443–50.

Daglish MR, Weinstein A, Malizia AL, Wilson S, Melichar JK, Britten S, Brewer C, Lingford-Hughes A, Myles JS, Grasby P, Nutt DJ. **2001**. Changes in regional cerebral blood flow elicited by craving memories in abstinent opiate-dependent subjects. *Am. J. Psychiatry* **158**: 1680–6.

Dajas-Bailador FA, Mogg AJ, Wonnacott S. **2002a**. Intracellular Ca2+ signals evoked by stimulation of nicotinic acetylcholine receptors in SH-SY5Y cells: contribution of voltage-operated Ca2+ channels and Ca2+ stores. *J. Neurochem.* **81**: 606–14.

Dajas-Bailador F a, Soliakov L, Wonnacott S. **2002b**. Nicotine activates the extracellular signal-regulated kinase 1/2 via the alpha7 nicotinic acetylcholine receptor and protein kinase A, in SH-SY5Y cells and hippocampal neurones. *J. Neurochem.* **80**: 520–30.

Dajas-Bailador F, Wonnacott S. **2004**. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends Pharmacol. Sci.* **25**: 317–24.

Dalley JW, Cardinal RN, Robbins TW. **2004**. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci. Biobehav. Rev.* **28**: 771–84.

Das P, Lilly SM, Zerda R, Gunning WT, Alvarez FJ, Tietz EI. **2008**. Increased AMPA receptor GluR1 subunit incorporation in rat hippocampal CA1 synapses during benzodiazepine withdrawal. *J. Comp. Neurol.* **511**: 832–46.

Davis HP, Squire LR. **1984**. Protein synthesis and memory: a review. *Psychol. Bull.* **96**: 518–59.

Decker MW, Brioni JD, Bannon AW, Arneric SP. **1995**. Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. *Life Sci.* **56**: 545–70.

Derkach V, Barria A, Soderling TR. **1999**. Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc. Natl. Acad. Sci. U. S. A.* **96**: 3269–74.

Derkach VA, Oh MC, Guire ES, Soderling TR. **2007**. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Rev. Neurosci.* **8**: 101–113.

Dickinson JA, Kew JNC, Wonnacott S. **2008**. Presynaptic _, 7- and ᴺL 2-Containing Nicotinic Acetylcholine Receptors Modulate Excitatory Amino Acid Release from Rat Prefrontal Cortex Nerve Terminals via Distinct Cellular Mechanisms. *Mol. Pharmacol.* **74**: 348–359.

Dityatev A, Dityateva G, Schachner M. **2000**. Synaptic strength as a function of post- versus presynaptic expression of the neural cell adhesion molecule NCAM. *Neuron* **26**: 207–17.

Dolorfo CL, Amaral DG. **1998**. Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J. Comp. Neurol.* **398**: 25–48.

Dominguez del Toro E, Juiz JM, Peng X, Lindstrom J, Criado M. **1994**. Immunocytochemical localization of the alpha 7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. *J. Comp. Neurol.* **349**: 325–42.

Dong HW, Petrovich GD, Swanson LW. **2001**. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res. Brain Res. Rev.* **38**: 192–246.

Dong Y, Saal D, Thomas M, Faust R, Bonci a, Robinson T, Malenka RC. **2004**. Cocaine-induced potentiation of synaptic strength in dopamine neurons: behavioral correlates in GluRA(-/-) mice. *Proc. Natl. Acad. Sci. U. S. A.* **101**: 14282–7.

Dopico AM, Lovinger DM. **2009**. Acute alcohol action and desensitization of ligand-gated ion channels. *Pharmacol. Rev.* **61**: 98–114.

Duncan CP. **1949**. The retroactive effect of electroshock on learning. *J. Comp. Physiol. Psychol.* **42**: 32–44.

Duncan G, Miyamoto S, Gu H, Lieberman J, Koller B, Snouwaert J. **2002**. Alterations in regional brain metabolism in genetic and pharmacological models of reduced NMDA receptor function. *Brain Res.* **951**: 166–76.

Dunlop J, Lock T, Jow B, Sitzia F, Grauer S, Jow F, Kramer A, Bowlby MR, Randall A, Kowal D, Gilbert A, Comery TA, Larocque J, Soloveva V, Brown J, Roncarati R. **2009**. Old and new pharmacology: positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine(2B/C) receptor antagonist SB-206553 (3,5-dihydro-5-methyl-N-3-pyridinylbenzo[1,2-b:4,5-b']di pyrrole-1(2H)-carboxamide). *J. Pharmacol. Exp. Ther.* **328**: 766–76.

Dutar P, Bassant MH, Senut MC, Lamour Y. **1995**. The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* **75**: 393–427.

Eisener-Dorman AF, Grabowski-Boase L, Tarantino LM. **2011**. Cocaine locomotor activation, sensitization and place preference in six inbred strains of mice. *Behav. Brain Funct.* **7**: 29.

Ericson M, Blomqvist O, Engel J a, Söderpalm B. **1998**. Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur. J. Pharmacol.* **358**: 189–96.

Ericson M, Löf E, Stomberg R, Chau P, Söderpalm B. **2008**. Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. *J. Pharmacol. Exp. Ther.* **326**: 76–82.

Exley R, Cragg SJ. **2008**. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br. J. Pharmacol.* **153 Suppl** : S283–S297.

Fabian-Fine R, Skehel P, Errington ML, Davies HA, Sher E, Stewart MG, Fine A. **2001**. Ultrastructural distribution of the alpha7 nicotinic acetylcholine receptor subunit in rat hippocampus. *J. Neurosci.* **21**: 7993–8003.

Fabian-Fine R, Volknandt W, Fine A, Stewart MG. **2000**. Age-dependent pre- and postsynaptic distribution of AMPA receptors at synapses in CA3 stratum radiatum of hippocampal slice cultures compared with intact brain. *Eur. J. Neurosci.* **12**: 3687–3700.

Famous KR, Kumaresan V, Sadri-Vakili G, Schmidt HD, Mierke DF, Cha J-HJ, Pierce RC. **2008**. Phosphorylation-dependent trafficking of GluR2-containing AMPA receptors in the nucleus accumbens plays a critical role in the reinstatement of cocaine seeking. *J. Neurosci.* **28**: 11061–70.

Fanselow MS, Dong H-W. **2010**. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* **65**: 7–19.

Farahmandfar M, Karimian SM, Zarrindast M-R, Kadivar M, Afrouzi H, Naghdi N. **2011a**. Morphine sensitization increases the extracellular level of glutamate in CA1 of rat hippocampus via μ-opioid receptor. *Neurosci. Lett.* **494**: 130–4.

Farahmandfar M, Zarrindast M-R, Kadivar M, Karimian SM, Naghdi N. **2011b**. The effect of morphine sensitization on extracellular concentrations of GABA in dorsal hippocampus of male rats. *Eur. J. Pharmacol.* **669**: 66–70.

Fasoli F, Gotti C. **2015**. Structure of neuronal nicotinic receptors. *Curr. Top. Behav. Neurosci.* **23**: 1–17.

Felix R, Levin ED. **1997**. Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience* **81**: 1009–1017.

Feng B, Xing J, Jia D, Liu S, Guo H, Li X, He X, Zhao M. **2011**. Blocking α4β2 and α7 nicotinic acetylcholine receptors inhibits the reinstatement of morphine-induced CPP by drug priming in mice. *Behav. Brain Res.* **220**: 100–5.

Fenster CP, Rains MF, Noerager B, Quick MW, Lester R a. **1997**. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J. Neurosci.* **17**: 5747–59.

Ferbinteanu J, McDonald RJ. **2001**. Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus* **11**: 187–200.

Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME. **2010**. The role of glutamate receptor redistribution in locomotor sensitization to cocaine. *Neuropsychopharmacology* **35**: 818–33.

Ferrario CR, Li X, Wolf ME. **2011**. Effects of acute cocaine or dopamine receptor agonists on AMPA receptor distribution in the rat nucleus accumbens. *Synapse* **65**: 54–63.

Feuerbach D, Nozulak J, Lingenhoehl K, McAllister K, Hoyer D. **2007**. JN403, in vitro characterization of a novel nicotinic acetylcholine receptor alpha7 selective agonist. *Neurosci. Lett.* **416**: 61–5.

Fiserová M, Consolo S, Krsiak M. **1999**. Chronic morphine induces long-lasting changes in acetylcholine release in rat nucleus accumbens core and shell: an in vivo microdialysis study. *Psychopharmacology (Berl).* **142**: 85–94.

Fitzgerald L, Ortiz J, Hamedani A, Nestler E. **1996**. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J. Neurosci.* **16**: 274–282.

Flexner LB, Flexner JB, Stellar E. **1965**. Memory and cerebral protein synthesis in mice as affected by graded amounts of puromycin. *Exp. Neurol.* **13**: 264–272.

Forster GL, Blaha CD. **2000**. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur. J. Neurosci.* **12**: 3596–3604.

Francis MM, Papke RL. **1996**. Muscle-type nicotinic acetylcholine receptor delta subunit determines sensitivity to noncompetitive inhibitors, while gamma subunit regulates divalent permeability. *Neuropharmacology* **35**: 1547–1556.

Frankland P, Bontempi B, Talton L, Kaczmarek L, Silva a. **2004**. The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science (80-.).* **304**: 881–883.

Franklin K, Paxinos G. **1997**. The mouse brain in stereotaxic coordinates. London: Academic Press.

Frazier CJ, Buhler a V, Weiner JL, Dunwiddie T V. **1998**. Synaptic potentials mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *J. Neurosci.* **18**: 8228–8235.

Freund TF, Buzsáki G. **1998**. Interneurons of the hippocampus. *Hippocampus* **6**: 347–470.

Fritz M, El Rawas R, Salti A, Klement S, Bardo MT, Kemmler G, Dechant G, Saria A, Zernig G. **2011**. Reversal of cocaine-conditioned place preference and mesocorticolimbic Zif268 expression by social interaction in rats. *Addict. Biol.* **16**: 273–84.

Frysztak RJ, Neafsey EJ. **1994**. The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. *Brain Res.* **643**: 181–93.

Fuchs JL. **1989**. [125I]alpha-bungarotoxin binding marks primary sensory area developing rat neocortex. *Brain Res.* **501**: 223–34.

Fucile S, Sucapane A, Eusebi F. **2005**. Ca2+ permeability of nicotinic acetylcholine receptors from rat dorsal root ganglion neurones. *J. Physiol.* **565**: 219–28.

Fujii S, Ji Z, Morita N, Sumikawa K. **1999**. Acute and chronic nicotine exposure differentially facilitate the induction of LTP. *Brain Res.* **846**: 137–43.

Fujii S, Sumikawa K. **2001**. Nicotine accelerates reversal of long-term potentiation and enhances long-term depression in the rat hippocampal CA1 region. *Brain Res.* **894**: 340–6.

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA. **2000**. Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. *Am. J. Psychiatry* **157**: 1789–98.

Garris PA, Kilpatrick M, Bunin MA. **1999**. Dissociation of dopamine release in the nucleus accumbens from intracranial. **8569**: 67–69.

Gawel K, Labuz K, Jenda M, Silberring J, Kotlinska JH. **2014**. Influence of cholinesterase inhibitors, donepezil and rivastigmine on the acquisition, expression, and reinstatement of morphine-induced conditioned place preference in rats. *Behav. Brain Res.* **268**: 169–76.

Gerdjikov T V, Ross GM, Beninger RJ. **2004**. Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behav. Neurosci.* **118**: 740–50.

Girden E, Culler E. **1937**. Conditioned responses in curaized striate muscle in dogs. *Journal of comparative Psychology.* **23(2)**:261-274

Glick SD, Maisonneuve IM, Kitchen B a, Fleck MW. **2002**. Antagonism of alpha 3 beta 4 nicotinic receptors as a strategy to reduce opioid and stimulant self-administration. *Eur. J. Pharmacol.* **438**: 99–105.

Goldberg JA, Reynolds JNJ. **2011**. Spontaneous firing and evoked pauses in the tonically active cholinergic interneurons of the striatum. *Neuroscience* **198**: 27–43.

Gotti C, Clementi F. **2004**. Neuronal nicotinic receptors: from structure to pathology. *Prog. Neurobiol.* **74**: 363–96.

Gotti C, Hanke W, Maury K, Moretti M, Ballivet M, Clementi F, Bertrand D. **1994**. Pharmacology and biophysical properties of alpha 7 and alpha 7-alpha 8 alpha-bungarotoxin receptor subtypes immunopurified from the chick optic lobe. *Eur. J. Neurosci.* **6**: 1281–91.

Gotti C, Riganti L, Vailati S, Clementi F. **2006a**. Brain neuronal nicotinic receptors as new targets for drug discovery. *Curr. Pharm. Des.* **12**: 407–28.

Gotti C, Zoli M, Clementi F. **2006b**. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* **27**: 482–91.

Gould RW, Czoty PW, Nader SH, Nader M a. **2011**. Effects of varenicline on the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys. *J. Pharmacol. Exp. Ther.* **339**: 678–86.

Grabus SD, Martin BR, Brown SE, Damaj MI. **2006**. Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. *Psychopharmacology (Berl).* **184**: 456–63.

Grady S, Marks MJ, Wonnacott S, Collins AC. **1992**. Characterization of nicotinic receptor-mediated [3H]dopamine release from synaptosomes prepared from mouse striatum. *J. Neurochem.* **59**: 848–56.

Griffin AL, Asaka Y, Darling RD, Berry SD. **2004**. Theta-contingent trial presentation accelerates learning rate and enhances hippocampal plasticity during trace eyeblink conditioning. *Behav. Neurosci.* **118**: 403–11.

Van Groen T, Wyss JM. **1990**. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J. Comp. Neurol.* **302**: 515–28.

Van Groen T, Wyss JM. **2003**. Connections of the retrosplenial granular b cortex in the rat. *J. Comp. Neurol.* **463**: 249–263.

Groenewegen HJ, Wright CI, Beijer A V. **1996**. The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog. Brain Res.* **107**: 485–511.

Grønlien JH, Håkerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J. **2007**. Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol. Pharmacol.* **72**: 715–24.

Gu Z, Lamb PW, Yakel JL. **2012**. Cholinergic coordination of presynaptic and postsynaptic activity induces timing-dependent hippocampal synaptic plasticity. *J. Neurosci.* **32**: 12337–48.

Gu Z, Yakel JL. **2011**. Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron* **71**: 155–65.

Guillem K, Peoples LL. **2010**. Varenicline effects on cocaine self administration and reinstatement behavior. *Behav. Pharmacol.* **21**: 96–103.

Gulledge AT, Park SB, Kawaguchi Y, Stuart GJ. **2007**. Heterogeneity of phasic cholinergic signaling in neocortical neurons. *J. Neurophysiol.* **97**: 2215–29.

Gunter C, Dhand R. **2002**. Human biology by proxy. *Nature* **420**: 509–509.

Guo Y, Wang H-L, Xiang X-H, Zhao Y. **2009**. The role of glutamate and its receptors in mesocorticolimbic dopaminergic regions in opioid addiction. *Neurosci. Biobehav. Rev.* **33**: 864–73.

Hahn B, Shoaib M, Stolerman IP. **2003**. Involvement of the prefrontal cortex but not the dorsal hippocampus in the attention-enhancing effects of nicotine in rats. *Psychopharmacology (Berl).* **168**: 271–279.

Hahn B, Shoaib M, Stolerman IP. **2011**. Selective nicotinic receptor antagonists: Effects on attention and nicotine-induced attentional enhancement. *Psychopharmacology (Berl).* **217**: 75–82.

Hajós M, Hurst RS, Hoffmann WE, Krause M, Wall TM, Higdon NR, Groppi VE. **2005**. The selective alpha7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide

hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats. *J. Pharmacol. Exp. Ther.* **312**: 1213–22.

Hallanger AE, Wainer BH. **1988**. Ascending projections from the pedunculopontine tegmental nucleus and the adjacent mesopontine tegmentum in the rat. *J. Comp. Neurol.* **274**: 483–515.

Han CJ, O'Tuathaigh CM, van Trigt L, Quinn JJ, Fanselow MS, Mongeau R, Koch C, Anderson DJ. **2003**. Trace but not delay fear conditioning requires attention and the anterior cingulate cortex. *Proc. Natl. Acad. Sci. U. S. A.* **100**: 13087–13092.

Hao Y, Yang J, Sun J, Qi J, Dong Y, Wu CF. **2008**. Lesions of the medial prefrontal cortex prevent the acquisition but not reinstatement of morphine-induced conditioned place preference in mice. *Neurosci. Lett.* **433**: 48–53.

Harris GC, Wimmer M, Byrne R, Aston-Jones G. **2004**. Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neuroscience* **129**: 841–7.

Hart N, Rocha A, Miller DK, Nation JR. **2010**. Dose dependent Attenuation of heroin Self-administration with Lobeline. **24**: 51–55.

Hashimoto K, Ishima T, Fujita Y, Matsuo M, Kobashi T, Takahagi M, Tsukada H, Iyo M. **2008**. Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the novel selective alpha7 nicotinic receptor agonist SSR180711. *Biol. Psychiatry* **63**: 92–7.

Health and Social Care Information Centre. **2012**. Statistics on Alcohol :England, 2012. *Heal. Soc. Care Inf. Cent.*

Hebb D. **1949**. The organisation of behaviour: a neuropsychological theory. Wiley: New York.

Hefft S, Hulo S, Bertrand D, Muller D. **1999**. Synaptic transmission at nicotinic acetylcholine receptors in rat hippocampal organotypic cultures and slices. *J.Physiol.* **515**: 769–776.

Heinrichs SC, Leite-Morris K a, Carey RJ, Kaplan GB. **2010**. Baclofen enhances extinction of opiate conditioned place preference. *Behav. Brain Res.* **207**: 353–9.

Henke PG. **1990**. Hippocampal pathway to the amygdala and stress ulcer development. *Brain Res. Bull.* **25**: 691–5.

Hernandez CM, Cortez I, Gu Z, Colón-Sáez JO, Lamb PW, Wakamiya M, Yakel JL, Dineley KT. **2014**. Research tool: Validation of floxed α7 nicotinic

acetylcholine receptor conditional knockout mice using in vitro and in vivo approaches. *J. Physiol.* **592**: 3201–14.

Higgins ST, Heil SH, Lussier JP. **2004**. Clinical implications of reinforcement as a determinant of substance use disorders. *Annu. Rev. Psychol.* **55**: 431–61.

Hill JJ, Zoli M, Bourgeois J, Changeux J. **1993**. Immunocytochemical localization of a neuronal nicotinic receptor: the beta 2-subunit. *J. Neurosci.* **13**: 1551–1568.

Hills T, Brockie PJ, Maricq A V. **2004**. Dopamine and glutamate control area-restricted search behavior in Caenorhabditis elegans. *J. Neurosci.* **24**: 1217–25.

Hoover WB, Vertes RP. **2007**. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct. Funct.* **212**: 149–79.

Hopkins TJ, Rupprecht LE, Hayes MR, Blendy J a, Schmidt HD. **2012**. Galantamine, an Acetylcholinesterase Inhibitor and Positive Allosteric Modulator of Nicotinic Acetylcholine Receptors, Attenuates Nicotine Taking and Seeking in Rats. *Neuropsychopharmacology* **37**: 2310–2321.

Hou Y, Liu Y, Kang S, Yu C, Chi Z, Liu J. **2009**. Glutamate receptors in the dorsal hippocampus mediate the acquisition, but not the expression, of conditioned place aversion induced by acute morphine withdrawal in rats. *Acta Pharmacol. Sin.* **30**: 1385–1391.

Le Houezec J, Halliday R, Benowitz NL, Callaway E, Naylor H, Herzig K. **1994**. A low dose of subcutaneous nicotine improves information processing in non-smokers. *Psychopharmacology (Berl).* **114**: 628–34.

Hu M. **2002**. Nicotinic Regulation of CREB Activation in Hippocampal Neurons by Glutamatergic and Nonglutamatergic Pathways. *Mol. Cell. Neurosci.* **21**: 616–625.

Huang CC, Liang YC, Hsu KS. **1999**. A role for extracellular adenosine in time-dependent reversal of long-term potentiation by low-frequency stimulation at hippocampal CA1 synapses. *J. Neurosci.* **19**: 9728–38.

Hunter BE, de Fiebre CM, Papke RL, Kem WR, Meyer EM. **1994**. A novel nicotinic agonist facilitates induction of long-term potentiation in the rat hippocampus. *Neurosci. Lett.* **168**: 130–4.

Hurst RS, Hajós M, Raggenbass M, Wall TM, Higdon NR, Lawson J a, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, Groppi VE, Allaman G, Ogier R, Bertrand S, Bertrand D, Arneric SP. **2005**. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J. Neurosci.* **25**: 4396–405.

Hyman SE, Malenka RC. **2001**. Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat. Rev. Neurosci.* **2**: 695–703.

Imoto K, Busch C, Sakmann B, Mishina M, Konno T, Nakai J, Bujo H, Mori Y, Fukuda K, Numa S. **1988**. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* **335**: 645–8.

Imperato A, Dazzi L, Carta G, Colombo G, Biggio G. **1998**. Rapid increase in basal acetylcholine release in the hippocampus of freely moving rats induced by withdrawal from long-term ethanol intoxication. *Brain Res.* **784**: 347–50.

Inglis WL, Olmstead MC, Robbins TW. **2000**. Pedunculopontine tegmental nucleus lesions impair stimulus--reward learning in autoshaping and conditioned reinforcement paradigms. *Behav. Neurosci.* **114**: 285–94.

Insel TR, Miller LP, Gelhard RE. **1990**. The ontogeny of excitatory amino acid receptors in rat forebrain—I.N-methyl-d-aspartate and quisqualate receptors. *Neuroscience* **35**: 31–43.

Ise Y, Narita M, Nagase H, Suzuki T. **2000**. Modulation of opioidergic system on mecamylamine-precipitated nicotine-withdrawal aversion in rats. *Psychopharmacology (Berl).* **151**: 49–54.

Ishikawa A, Nakamura S. **2006**. Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. *J. Neurophysiol.* **96**: 2134–8.

Istvan J, Matarazzo JD. **1984**. Tobacco, alcohol, and caffeine use: a review of their interrelationships. *Psychol. Bull.* **95**: 301–26.

Itzhak Y, Martin JL. **2002**. Cocaine-induced conditioned place preference in mice: Induction, extinction and reinstatement by related psychostimulants. *Neuropsychopharmacology* **26**: 130–134.

Jaffe JH. **1992**. Current concepts of addiction. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **70**: 1–21.

Jensen AA, Frølund B, Liljefors T, Krogsgaard-Larsen P. **2005**. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J. Med. Chem.* **48**: 4705–45.

Ji D, Dani J a. **2000**. Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J. Neurophysiol.* **83**: 2682–2690.

Ji D, Lape R, Dani JA. **2001a**. Timing and Location of Nicotinic Hippocampal Synaptic Plasticity. **31**: 131–141.

Ji D, Lape R, Dani JA. **2001b**. Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. *Neuron* **31**: 131–41.

Johanson C-E, Frey KA, Lundahl LH, Keenan P, Lockhart N, Roll J, Galloway GP, Koeppe RA, Kilbourn MR, Robbins T, Schuster CR. **2006**. Cognitive function and nigrostriatal markers in abstinent methamphetamine abusers. *Psychopharmacology (Berl).* **185**: 327–38.

Johnson SW, North R a. **1992**. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* **12**: 483–8.

Johnston D, Williams S, Jaffe D, Gray R. **1992**. NMDA-receptor-independent long-term potentiation. *Annu. Rev. Physiol.* **54**: 489–505.

Jolas T, Nestler EJ, Aghajanian GK. **2000**. Chronic morphine increases GABA tone on serotonergic neurons of the dorsal raphe nucleus: association with an up-regulation of the cyclic AMP pathway. *Neuroscience* **95**: 433–43.

Jones GH, Robbins TW. **1992**. Differential effects of mesocortical, mesolimbic, and mesostriatal dopamine depletion on spontaneous, conditioned, and drug-induced locomotor activity. *Pharmacol. Biochem. Behav.* **43**: 887–895.

Jones MW, Wilson MA. **2005**. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. *PLoS Biol.* **3**: e402.

Jung M, Wiener S, McNaughton B. **1994**. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J. Neurosci.* **14**: 7347–7356.

Kalivas PW, Duffy P, Eberhardt H. **1990**. Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J. Pharmacol. Exp. Ther.* **253**: 858–66.

Kalivas PW, Ph D, Volkow ND. **2005**. Reviews and Overviews The Neural Basis of Addiction : A Pathology of Motivation and Choice. *Psychiatry Interpers. Biol. Process.*: 1403–1413.

Kamens HM, Andersen J, Picciotto MR. **2010**. Modulation of ethanol consumption by genetic and pharmacological manipulation of nicotinic acetylcholine receptors in mice. *Psychopharmacology (Berl).* **208**: 613–26.

Kamens HM, Hoft NR, Cox RJ, Miyamoto JH, Ehringer M a. **2012**. The α6 nicotinic acetylcholine receptor subunit influences ethanol-induced sedation. *Alcohol* **46**: 463–71.

Kamens HM, Phillips TJ. **2008**. A role for neuronal nicotinic acetylcholine receptors in ethanol-induced stimulation, but not cocaine- or methamphetamine-induced stimulation. *Psychopharmacology (Berl).* **196**: 377–87.

Van Kampen M, Selbach K, Schneider R, Schiegel E, Boess F, Schreiber R. **2004**. AR-R 17779 improves social recognition in rats by activation of nicotinic alpha7 receptors. *Psychopharmacology (Berl).* **172**: 375–83.

Kargari A, Ramshini E, Alaei H, Sedighi M, Oryan S. **2012**. Different current intensities electrical stimulation of prelimbic cortex of mPFC produces different effects on morphine-induced conditioned place preference in rats. *Behav. Brain Res.* **231**: 187–92.

Karlin A. **1993**. Structure of nicotinic acetylcholine receptors. *Curr. Opin. Neurobiol.* **3**: 299–309.

Karlin A. **2002**. Emerging structure of the nicotinic acetylcholine receptors. *Nat. Rev. Neurosci.* **3**: 102–14.

Katz J, Halstead W. **1950**. Protein organization and mental function. *Comp. Psychol. Monogr.* **20**: 1–38.

Kauer J a, Malenka RC. **2007**. Synaptic plasticity and addiction. *Nat. Rev. Neurosci.* **8**: 844–58.

Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC. **1995**. Striatal interneurones: chemical, physiological and morphological characterization. *Trends Neurosci.* **18**: 527–35.

Kelley AE, Berridge KC. **2002**. The neuroscience of natural rewards: relevance to addictive drugs. *J. Neurosci.* **22**: 3306–11.

Kenney JW, Gould TJ. **2008**. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol. Neurobiol.* **38**: 101–21.

Keralapurath MM, Clark JK, Hammond S, Wagner JJ. **2014**. Cocaine- or stress-induced metaplasticity of LTP in the dorsal and ventral hippocampus. *Hippocampus* **24**: 577–590.

Kim E, Sheng M. **2004**. PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* **5**: 771–81.

Kitagawa H, Takenouchi T, Azuma R, Wesnes KA, Kramer WG, Clody DE, Burnett AL. **2003**. Safety, pharmacokinetics, and effects on cognitive function of multiple doses of GTS-21 in healthy, male volunteers. *Neuropsychopharmacology* **28**: 542–51.

Kobayashi Y, Amaral D. **2007**. Morphological characterization of spinal cord dorsal horn lamina I neurons projecting to the parabrachial nucleus in the rat. *J. Comp. Neurol.* **504**: 287–297.

Koob GF, Sanna PP, Bloom FE. **1998**. Neuroscience of Addiction Review. **21**: 467–476.

Kulak JM, Nguyen TA, Olivera BM, McIntosh JM. **1997**. Alpha-conotoxin MII blocks nicotine-stimulated dopamine release in rat striatal synaptosomes. *J. Neurosci.* **17**: 5263–70.

Kusayama T, Watanabe S. **2000**. Reinforcing effects of methamphetamine in planarians. *Neuroreport* **11**: 2511–3.

Kwatra MM, Benovic JL, Caron MG, Lefkowitz RJ, Hosey MM. **1989**. Phosphorylation of chick heart muscarinic cholinergic receptors by the beta-adrenergic receptor kinase. *Biochemistry* **28**: 4543–7.

Lacagnina M. **2015**. Neuroimmune signaling and opioids: impact of early-life experience on glial function and addiction-related behaviors. INRC, Phoenix.

Lagostena L, Trocme-Thibierge C, Morain P, Cherubini E. **2008**. The partial alpha7 nicotine acetylcholine receptor agonist S 24795 enhances long-term potentiation at CA3-CA1 synapses in the adult mouse hippocampus. *Neuropharmacology* **54**: 676–85.

Larson J, Xiao P, Lynch G. **1993**. Reversal of LTP by theta frequency stimulation. *Brain Res.* **600**: 97–102.

Larsson A, Edström L, Svensson L, Söderpalm B, Engel JA. **2005**. Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat. *Alcohol Alcohol* **40**: 349–58.

Larsson A, Svensson L, Söderpalm B, Engel J a. **2002**. Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. *Alcohol* **28**: 157–67.

Lavenex PB, Amaral DG, Lavenex P. **2006**. Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. *J. Neurosci.* **26**: 4546–4558.

Laviola G, Dell'Omo G, Alleva E, Bignami G. **1992**. Ontogeny of cocaine hyperactivity and conditioned place preference in mice. *Psychopharmacology (Berl).* **107**: 221–228.

Laviolette SR, van der Kooy D. **2003**. The motivational valence of nicotine in the rat ventral tegmental area is switched from rewarding to aversive following blockade of the alpha7-subunit-containing nicotinic acetylcholine receptor. *Psychopharmacology (Berl).* **166**: 306–13.

Lavoie B, Parent A. **1994**. Pedunculopontine nucleus in the squirrel monkey: cholinergic and glutamatergic projections to the substantia nigra. *J. Comp. Neurol.* **344**: 232–41.

Lê a D, Corrigall W a, Harding JW, Juzytsch W, Li TK. **2000**. Involvement of nicotinic receptors in alcohol self-administration. *Alcohol. Clin. Exp. Res.* **24**: 155–163.

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL. **2000**. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**: 955–9.

Legault M, Wise RA. **2001**. Novelty-evoked elevations of nucleus accumbens dopamine: dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental area. *Eur. J. Neurosci.* **13**: 819–28.

Leonard RJ, Labarca CG, Charnet P, Davidson N, Lester HA. **1988**. Evidence that the M2 membrane-spanning region lines the ion channel pore of the nicotinic receptor. *Science* **242**: 1578–81.

Lesage MG, Stafford D, Glowa JR. **1999**. Preclinical research on cocaine self-administration: Environmental determinants and their interaction with pharmacological treatment. *Neurosci. Biobehav. Rev.* **23**: 717–741.

Levin ED. **2002**. Nicotinic receptor subtypes and cognitive function. *J. Neurobiol.* **53**: 633–40.

Levin ED. **2013**. Complex relationships of nicotinic receptor actions and cognitive functions. *Biochem. Pharmacol.* **86**: 1145–52.

Levin ED, Bettegowda C, Blosser J, Gordon J. **1999**. AR-R17779, and alpha7 nicotinic agonist, improves learning and memory in rats. *Behav. Pharmacol.* **10**: 675–80.

Levin ED, Conners CK, Sparrow E, Hinton SC, Erhardt D, Meck WH, Rose JE, March J. **1996a**. Nicotine effects on adults with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl).* **123**: 55–63.

Levin ED, Mead T, Rezvani a H, Rose JE, Gallivan C, Gross R. **2000**. The nicotinic antagonist mecamylamine preferentially inhibits cocaine vs. food self-administration in rats. *Physiol. Behav.* **71**: 565–70.

Levin ED, Rezvani AH, Xiao Y, Slade S, Cauley M, Wells C, Hampton D, Petro A, Rose JE, Brown ML, Paige M a, McDowell BE, Kellar KJ. **2010**. Sazetidine-A, a selective alpha4beta2 nicotinic receptor desensitizing agent and partial agonist, reduces nicotine self-administration in rats. *J. Pharmacol. Exp. Ther.* **332**: 933–939.

Levin ED, Simon BB. **1998**. Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl).* **138**: 217–30.

Levin ED, Wilson W, Rose JE, McEvoy J. **1996b**. Nicotine-haloperidol interactions and cognitive performance in schizophrenics. *Neuropsychopharmacology* **15**: 429–36.

Li F, Wang XS, Dai RP, Zhang JY, Zhou XF, Hao W, Li CQ. **2011**. The activation of NMDA receptor-ERK pathway in the central amygdala is required for the expression of morphine-conditioned place preference in the rat. *Neurotox. Res.* **20**: 362–371.

Lindstrom J. **1997**. Nicotinic acetylcholine receptors in health and disease. *Mol. Neurobiol.* **15**: 193–222.

Lindzey G, Winston H, Manosevitz M. **1961**. Social dominance in inbred mouse strains. *Subj. Strain Bibliogr. 1961*: 474 – 476.

Lisman JE, Grace A a. **2005**. The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron* **46**: 703–713.

Lisman J, Raghavachari S. **2006**. A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses. *Sci. STKE* **2006**: re11.

Liu X, Caggiula AR, Yee SK, Nobuta H, Sved AF, Pechnick RN, Poland RE. **2007**. Mecamylamine Attenuates Cue-Induced Reinstatement of Nicotine-Seeking Behavior in Rats. *Neuropsychopharmacology* **32**: 710–718.

Liu Q, Huang Y, Xue F, Simard A, DeChon J, Li G, Zhang J, Lucero L, Wang M, Sierks M, Hu G, Chang Y, Lukas RJ, Wu J. **2009**. A novel nicotinic acetylcholine receptor subtype in basal forebrain cholinergic neurons with high sensitivity to amyloid peptides. *J. Neurosci.* **29**: 918–29.

Liu S, Zheng D, Peng X-X, Cabeza de Vaca S, Carr KD. **2011**. Enhanced cocaine-conditioned place preference and associated brain regional levels of BDNF, p-ERK1/2 and p-Ser845-GluA1 in food-restricted rats. *Brain Res.* **1400**: 31–41.

Livingstone PD, Dickinson J a, Srinivasan J, Kew JNC, Wonnacott S. **2010**. Glutamate-dopamine crosstalk in the rat prefrontal cortex is modulated by Alpha7 nicotinic receptors and potentiated by PNU-120596. *J. Mol. Neurosci.* **40**: 172–6.

Loimer N, Vedovelli H, Presslich O, Werner E, Hollerer E, Pferdmann V, Schmidsiegel B. **1991**. Nicotine drug addiction aids drug taking characteristics of viennese addicts. *Wien. Klin. Wochenschr.* **103**: 617–620.

Lopez-Hernandez G, Placzek AN, Thinschmidt JS, Lestage P, Trocme-Thibierge C, Morain P, Papke RL. **2007**. Partial agonist and neuromodulatory activity of S 24795 for alpha7 nAChR responses of hippocampal interneurons. *Neuropharmacology* **53**: 134–44.

Lu L, Xu NJ, Ge X, Yue W, Su WJ, Pei G, Ma L. **2002**. Reactivation of morphine conditioned place preference by drug priming: Role of environmental cues and sensitization. *Psychopharmacology (Berl).* **159**: 125–132.

Lu L, Zeng S, Liu D, Ceng X. **2000**. Inhibition of the amygdala and hippocampal calcium/calmodulin-dependent protein kinase II attenuates the dependence and relapse to morphine differently in rats. *Neurosci. Lett.* **291**: 191–195.

Luo A, Tahsili-Fahadan P, Wise RA, Lupica CR, Aston-Jones G. **2011**. Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. *Science (80-.).* **333**: 353–357.

Lynch G. **2002**. Memory enhancement: the search for mechanism-based drugs. *Nat. Neurosci.* **5**: 1035–8.

Ma Y-Y, Chu N-N, Guo C-Y, Han J-S, Cui C-L. **2007**. NR2B-containing NMDA receptor is required for morphine-but not stress-induced reinstatement. *Exp. Neurol.* **203**: 309–19.

Ma Y-Y, Guo C-Y, Yu P, Lee DY-W, Han J-S, Cui C-L. **2006**. The role of NR2B containing NMDA receptor in place preference conditioned with morphine and natural reinforcers in rats. *Exp. Neurol.* **200**: 343–55.

Macdermott AB, Role LW, Siegelbaum SA. **1999**. Presynaptic ionotrophic receptors and the control of transmitter release. *Annu. Rev. Neurosci.* **22**: 443–85.

Maldonado C, Rodríguez-Arias M, Castillo a, Aguilar M a, Miñarro J. **2007**. Effect of memantine and CNQX in the acquisition, expression and reinstatement of cocaine-induced conditioned place preference. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **31**: 932–9.

Malenka RC. **1999**. Long-Term Potentiation--A Decade of Progress? *Science (80-.).* **285**: 1870–1874.

Malinow R, Mainen ZF, Hayashi Y. **2000**. LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.* **10**: 352–7.

Man H-Y, Sekine-Aizawa Y, Huganir RL. **2007**. Regulation of -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. *Proc. Natl. Acad. Sci.* **104**: 3579–3584.

Manzanedo C, Aguilar MA, Rodríguez-Arias M, Miñarro J. **2001**. Effects of dopamine antagonists with different receptor blockade profiles on morphine-induced place preference in male mice. *Behav. Brain Res.* **121**: 189–97.

Marighetto A, Valerio S, Desmedt A, Philippin JN, Trocmé-Thibierge C, Morain P. **2008**. Comparative effects of the alpha7 nicotinic partial agonist, S 24795, and the cholinesterase inhibitor, donepezil, against aging-related deficits in declarative and working memory in mice. *Psychopharmacology (Berl).* **197**: 499–508.

Marks MJ, Whiteaker P, Calcaterra J, Stitzel JA, Bullock AE, Grady SR, Picciotto MR, Changeux JP, Collins AC. **1999**. Two pharmacologically distinct components of nicotinic receptor-mediated rubidium efflux in mouse brain require the beta2 subunit. *J. Pharmacol. Exp. Ther.* **289**: 1090–103.

Martí Barros D, Ramirez MR, Dos Reis EA, Izquierdo I. **2004**. Participation of hippocampal nicotinic receptors in acquisition, consolidation and retrieval of memory for one trial inhibitory avoidance in rats. *Neuroscience* **126**: 651–6.

Martin BR, Aceto MD. **1981**. Nicotine binding sites and their localization in the central nervous system. *Neurosci. Biobehav. Rev.* **5**: 473–8.

Martin-Soelch C, Chevalley a. F, Künig G, Missimer J, Magyar S, Mino a., Schultz W, Leenders KL. **2001**. Changes in reward-induced brain activation in opiate addicts. *Eur. J. Neurosci.* **14**: 1360–1368.

Martin-Sölch C, Magyar S, Künig G, Missimer J, Schultz W, Leenders KL. **2001**. Changes in brain activation associated with reward processing in smokers and nonsmokers. A positron emission tomography study. *Exp. brain Res.* **139**: 278–86.

Martínez-Hernández J, Lanuza E, Martínez-García F. **2012**. Lesions of the dopaminergic innervation of the nucleus accumbens medial shell delay the generation of preference for sucrose, but not of sexual pheromones. *Behav. Brain Res.* **226**: 538–47.

Marubio LM, Changeux J. **2000**. Nicotinic acetylcholine receptor knockout mice as animal models for studying receptor function. *Eur. J. Pharmacol.* **393**: 113–21.

Matsuyama S, Matsumoto a, Enomoto T, Nishizaki T. **2000**. Activation of nicotinic acetylcholine receptors induces long-term potentiation in vivo in the intact mouse dentate gyrus. *Eur. J. Neurosci.* **12**: 3741–7.

Mattson BJ, Morrell JI. **2005**. Preference for cocaine- versus pup-associated cues differentially activates neurons expressing either Fos or cocaine- and amphetamine-regulated transcript in lactating, maternal rodents. *Neuroscience* **135**: 315–28.

Maylie J, Adelman JP. **2010**. Cholinergic signaling through synaptic SK channels: it's a protein kinase but which one? *Neuron* **68**: 809–11.

McEown K, Treit D. **2010**. Inactivation of the dorsal or ventral hippocampus with muscimol differentially affects fear and memory. *Brain Res.* **1353**: 145–51.

McFarland K, Lapish CC, Kalivas PW. **2003**. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.* **23**: 3531–7.

McGaugh JL. **2004**. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci.* **27**: 1–28.

McGehee DS. **2002a**. Nicotinic receptors and hippocampal synaptic plasticity... It's all in the timing. *Trends Neurosci.* **25**: 171–172.

McGehee DS. **2002b**. Nicotinic receptors and hippocampal synaptic plasticity…it's all in the timing. *Trends Neurosci.* **25**: 171–172.

Mcgehee DS, Heath MJS, Gelber S, Devay P, Lorna W, Role LW. **1995**. Nicotine ehancement of fast synaptic transmission in CNS by presynaptic receptors. *Science (80-.).* **269**: 1692–1696.

McGehee DS, Role LW. **1995**. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu. Rev. Physiol.* **57**: 521–46.

McKay BE, Placzek AN, Dani J a. **2007**. Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **74**: 1120–33.

McLaughlin J, See RE. **2003**. Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology (Berl).* **168**: 57–65.

McQuiston a. R. **2014**. Acetylcholine release and inhibitory interneuron activity in hippocampal CA1. *Front. Synaptic Neurosci.* **6**: 1–7.

McQuiston a R, Madison D V. **1999**. Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. *J. Neurosci.* **19**: 2887–2896.

Mellgren SI, Srebro B. **1973**. Changes in acetylcholinesterase and distribution of degenerating fibres in the hippocampal region after septal lesions in the rat. *Brain Res.* **52**: 19–36.

Meyer EM, Tay ET, Papke RL, Meyers C, Huang GL, de Fiebre CM. **1997**. 3-[2,4-Dimethoxybenzylidene]anabaseine (DMXB) selectively activates rat alpha7 receptors and improves memory-related behaviors in a mecamylamine-sensitive manner. *Brain Res.* **768**: 49–56.

Meyers RA, Zavala AR, Neisewander JL. **2003**. Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning. *Neuroreport* **14**: 2127–31.

Mickiewicz AL, Napier TC. **2011**. Repeated exposure to morphine alters surface expression of AMPA receptors in the rat medial prefrontal cortex. *Eur. J. Neurosci.* **33**: 259–265.

Miczek K a, Thompson ML, Shuster L. **1985**. Naloxone injections into the periaqueductal grey area and arcuate nucleus block analgesia in defeated mice. *Psychopharmacology (Berl).* **87**: 39–42.

Millar NS, Gotti C. **2009**. Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology* **56**: 237–46.

Miller DK, Lever JR, Rodvelt KR, Baskett J a., Will MJ, Kracke GR. **2007**. Lobeline, a potential pharmacotherapy for drug addiction, binds to μ opioid receptors and diminishes the effects of opioid receptor agonists. *Drug Alcohol Depend.* **89**: 282–291.

Miller PS, Smart TG. **2010**. Binding, activation and modulation of Cys-loop receptors. *Trends Pharmacol. Sci.* **31**: 161–74.

Miyazawa A, Fujiyoshi Y, Unwin N. **2003**. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423**: 949–55.

Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, Wonnacott S. **2002**. Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. *J. Pharmacol. Exp. Ther.* **302**: 197–204.

Monaghan DT, Bridges RJ, Cotman CW. **1989**. Pharmacology , and Distinct Properties In System. *Ann. Rev. Pharmacol. Toxicol.*

Monaghan DT, Holets VR, Toy DW, Cotman CW. **1984a**. Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. *Nature* **306**: 176–9.

Monaghan DT, Yao D, Cotman CW. **1984b**. Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Res.* **324**: 160–164.

Moretti M, Zoli M, George AA, Lukas RJ, Pistillo F, Maskos U, Whiteaker P, Gotti C. **2014**. The novel α7β2-nicotinic acetylcholine receptor subtype is expressed in mouse and human basal forebrain: biochemical and pharmacological characterization. *Mol. Pharmacol.* **86**: 306–17.

Morgan D, Grant K a, Gage HD, Mach RH, Kaplan JR, Prioleau O, Nader SH, Buchheimer N, Ehrenkaufer RL, Nader M a. **2002**. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat. Neurosci.* **5**: 169–174.

Morón J a, Gullapalli S, Taylor C, Gupta A, Gomes I, Devi L a. **2010**. Modulation of opiate-related signaling molecules in morphine-dependent conditioned behavior: conditioned place preference to morphine induces CREB phosphorylation. *Neuropsychopharmacology* **35**: 955–66.

Morris RG, Garrud P, Rawlins JN, O'Keefe J. **1982**. Place navigation impaired in rats with hippocampal lesions. *Nature* **297**: 681–683.

Moser E, Moser MB, Andersen P. **1993**. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J. Neurosci.* **13**: 3916–3925.

Mucha RF, Van Der Kooy D, O'Shaughnessy M, Bucenieks P. **1982**. Drug reinforcement studied by the use of place conditioning in rat. *Brain Res.* **243**: 91–105.

Mueller D, Perdikaris D, Stewart J. **2002**. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. *Behav. Brain Res.* **136**: 389–97.

Mueller D, Stewart J. **2000**. Cocaine-induced conditioned place preference: Reinstatement by priming injections of cocaine after extinction. *Behav. Brain Res.* **115**: 39–47.

Mullen G, Napier J, Balestra M, DeCory T, Hale G, Macor J, Mack R, Loch J, Wu E, Kover A, Verhoest P, Sampognaro A, Phillips E, Zhu Y, Murray R, Griffith R, Blosser J, Gurley D, Machulskis A, Zongrone J, Rosen A, Gordon J. **2000**. (-)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor. *J. Med. Chem.* **43**: 4045–50.

Mustroph ML, Stobaugh DJ, Miller DS, Deyoung EK, Rhodes JS. **2011**. Wheel running can accelerate or delay extinction of conditioned place preference for cocaine in male C57BL/6J mice, depending on timing of wheel access. *Eur. J. Neurosci.* **34**: 1161–9.

Myers SJ, Dingledine R, Borges K. **1999**. Genetic regulation of glutamate receptor ion channels. *Annu. Rev. Pharmacol. Toxicol.* **39**: 221–241.

Naber P a, Witter MP. **1998**. Subicular efferents are organised mostly as parallel projections: A double-labeling, retrograde-tracing study in the rat. *J. Comp. Neurol.* **297**: 284–297.

Nai Q, McIntosh JM, Margiotta JF. **2003**. Relating neuronal nicotinic acetylcholine receptor subtypes defined by subunit composition and channel function. *Mol. Pharmacol.* **63**: 311–24.

Nakauchi S, Sumikawa K. **2014**. Endogenous ACh suppresses LTD induction and nicotine relieves the suppression via different nicotinic ACh receptor subtypes in the mouse hippocampus. *Life Sci.* **111**: 62–8.

Nakayama H, Numakawa T, Ikeuchi T, Hatanaka H. **2001**. Nicotine-induced phosphorylation of extracellular signal-regulated protein kinase and CREB in PC12h cells. *J. Neurochem.* **79**: 489–98.

Narita M, Funada M, Suzuki T. **2001**. Regulations of opioid dependence by opioid receptor types. *Pharmacol. Ther.* **89**: 1–15.

Nauen DW. **2011**. Methods of measuring activity at individual synapses: A review of techniques and the findings they have made possible. *J. Neurosci. Methods* **194**: 195–205.

Nestler EJ. **2001**. Molecular basis of long-term plasticity underlying addiction. *Nat. Rev. Neurosci.* **2**: 119–28.

Nestler E. **2002**. Common Molecular and Cellular Substrates of Addiction and Memory. *Neurobiol. Learn. Mem.* **78**: 637–647.

Neugebauer NM, Einstein EB, Lopez MB, McClure-Begley TD, Mineur YS, Picciotto MR. **2013**. Morphine dependence and withdrawal induced changes in cholinergic signaling. *Pharmacol. Biochem. Behav.* **109**: 77–83.

Newpher TM, Ehlers MD. **2008**. Glutamate receptor dynamics in dendritic microdomains. *Neuron* **58**: 472–97.

Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, Gee KW. **2007**. Nootropic alpha7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. *Proc. Natl. Acad. Sci. U. S. A.* **104**: 8059–64.

Nguyen P V, Kandel ER. **1996**. A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *J. Neurosci.* **16**: 3189–98.

Nibuya M, Morinobu S, Duman RS. **1995**. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* **15**: 7539–47.

Nicola SM, Surmeier J, Malenka RC. **2000**. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* **23**: 185–215.

Nicoll R a., Kauer J a., Malenka RC. **1988**. The current excitement in long term potentiation. *Neuron* **1**: 97–103.

Nirogi R, Kandikere V, Bhyrapuneni G, Saralaya R, Muddana N, Komarneni P. **2012**. Methyllycaconitine: A non-radiolabeled ligand for mapping α7 neuronal nicotinic acetylcholine receptors - In vivo target localization and biodistribution in rat brain. *J. Pharmacol. Toxicol. Methods* **66**: 22–8.

Nisell M, Nomikos GG, Svensson TH. **1994**. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse* **16**: 36–44.

Nishizaki T, Matsuoka T, Nomura T, Matsuyama S, Watabe S, Shiotani T, Yoshii M. **1999**. A ‘long-term-potentiation-like’ facilitation of hippocampal synaptic transmission induced by the nootropic nefiracetam. *Brain Res.* **826**: 281–8.

Nott A, Levin ED. **2006**. Dorsal hippocampal α7 and α4β2 nicotinic receptors and memory. *Brain Res.* **1081**: 72–78.

Le Novère N, Changeux JP. **1995**. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. *J. Mol. Evol.* **40**: 155–72.

Le Novère N, Zoli M, Changeux JP. **1996**. Neuronal nicotinic receptor alpha 6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur. J. Neurosci.* **8**: 2428–39.

Nutt DJ, King L a., Phillips LD. **2010**. Drug harms in the UK: A multicriteria decision analysis. *Lancet* **376**: 1558–1565.

Nyakas C, Luiten PG, Spencer DG, Traber J. **1987**. Detailed projection patterns of septal and diagonal band efferents to the hippocampus in the rat with emphasis on innervation of CA1 and dentate gyrus. *Brain Res. Bull.* **18**: 533–45.

Van den Oever MC, Goriounova N a, Li KW, Van der Schors RC, Binnekade R, Schoffelmeer ANM, Mansvelder HD, Smit AB, Spijker S, De Vries TJ. **2008**. Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. *Nat. Neurosci.* **11**: 1053–8.

Van den Oever MC, Lubbers BR, Goriounova N a, Li KW, Van der Schors RC, Loos M, Riga D, Wiskerke J, Binnekade R, Stegeman M, Schoffelmeer ANM, Mansvelder HD, Smit AB, De Vries TJ, Spijker S. **2010**. Extracellular Matrix Plasticity and GABAergic Inhibition of Prefrontal Cortex Pyramidal Cells Facilitates Relapse to Heroin Seeking. *Neuropsychopharmacology* **35**: 2120–2133.

Ohno M, Yamamoto T, Watanabe S. **1993**. Blockade of hippocampal nicotinic receptors impairs working memory but not reference memory in rats. *Pharmacol. Biochem. Behav.* **45**: 89–93.

Okabe S. **2007**. Molecular anatomy of the postsynaptic density. *Mol. Cell. Neurosci.* **34**: 503–18.

Okamoto T, Harnett MT, Morikawa H. **2006**. Hyperpolarization-activated cation current (Ih) is an ethanol target in midbrain dopamine neurons of mice. *J. Neurophysiol.* **95**: 619–26.

Oliverio A, Castellano C. **1974**. Experience modifies morphine-induced behavioral excitation of mice. *Nature* **252**: 229–30.

Olsen RW, Szamraj O, Houser CR. **1987**. [3H]AMPA binding to glutamate receptor subpopulations in rat brain. *Brain Res.* **402**: 243–254.

Olson VG, Zabetian CP, Bolanos C a, Edwards S, Barrot M, Eisch AJ, Hughes T, Self DW, Neve RL, Nestler EJ. **2005**. Regulation of drug reward by cAMP response element-binding protein: evidence for two functionally distinct subregions of the ventral tegmental area. *J. Neurosci.* **25**: 5553–62.

Olton DS, Collison C, Werz MA. **1977**. Spatial memory and radial arm maze performance of rats. *Learn. Motiv.* **8**: 289–314.

Opazo P, Choquet D. **2011**. A three-step model for the synaptic recruitment of AMPA receptors. *Mol. Cell. Neurosci.* **46**: 1–8.

Orlandi C, La Via L, Bonini D, Mora C, Russo I, Barbon A, Barlati S. **2011**. AMPA receptor regulation at the mRNA and protein level in rat primary cortical cultures. *PLoS One* **6**: 1–12.

Orr-Urtreger A, Göldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW, Beaudet AL. **1997**. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J. Neurosci.* **17**: 9165–71.

Orsetti M, Casamenti F, Pepeu G. **1996**. Enhanced acetylcholine release in the hippocampus and cortex during acquisition of an operant behavior. *Brain Res.* **724**: 89–96.

Overton D. **1978**. Major theories od state dependent learning. In: Ho B, Richards D, Chute D, eds. Drug discrimination and state dependent learning. Academic Press: New York, 283–318.

Ozawa S. **1998**. Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* **54**: 581–618.

Palomero-Gallagher N, Bidmon HJ, Zilles K. **2003**. AMPA, kainate, and NMDA receptor densities in the hippocampus of untreated male rats and females in estrus and diestrus. *J. Comp. Neurol.* **459**: 468–474.

Pan W-X, Schmidt R, Wickens JR, Hyland BI. **2005**. Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. *J. Neurosci.* **25**: 6235–6242.

Panagis G, Kastellakis a, Spyraki C, Nomikos G. **2000**. Effects of methyllycaconitine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology (Berl).* **149**: 388–96.

Pandis C, Sotiriou E, Kouvaras E, Asprodini E, Papatheodoropoulos C, Angelatou F. **2006**. Differential expression of NMDA and AMPA receptor subunits in rat dorsal and ventral hippocampus. *Neuroscience* **140**: 163–75.

Panos JJ, Rademacher DJ, Renner SL, Steinpreis RE. **1999**. The rewarding properties of NMDA and MK-801 (dizocilpine) as indexed by the conditioned place preference paradigm. *Pharmacol. Biochem. Behav.* **64**: 591–5.

Papke RL, Sanberg PR, Shytle RD. **2001**. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. *J. Pharmacol. Exp. Ther.* **297**: 646–56.

Paredes RG. **2009**. Evaluating the neurobiology of sexual reward. *ILAR J.* **50**: 15–27.

Parikh V, Pomerleau F, Huettl P, Gerhardt GA, Sarter M, Bruno JP. **2004**. Rapid assessment of in vivo cholinergic transmission by amperometric detection of changes in extracellular choline levels. *Eur. J. Neurosci.* **20**: 1545–54.

Park W-K, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC. **2002**. Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J. Neurosci.* **22**: 2916–25.

Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. **2006**. Storage of spatial information by the maintenance mechanism of LTP. *Science* **313**: 1141–1144.

Patrick J, Boulter J, Deneris E, Wada K, Wada E, Connolly J, Swanson L, Heinemann S. **1989**. Structure and function of neuronal nicotinic acetylcholine receptors deduced from cDNA clones. *Prog. Brain Res.* **79**: 27–33.

Paul S, Jeon WK, Bizon JL, Han J-S. **2015**. Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment. *Front. Aging Neurosci.* **7**: 1–11.

Paulsen O, Moser EI. **1998**. A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends Neurosci.* **21**: 273–8.

Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. **1998**. Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. *Learn. Mem.* **5**: 302–16.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. Elsevier, London

Pearson RCA, Sofroniew MV, Powell TPS. **1987**. The cholinergic nuclei of the basal forebrain of the rat: hypertrophy following contralateral cortical damage or section of the corpus callosum. *Brain Res.* **411**: 332–340.

Perkel BYDJ, Nicoll RA. **1993**. Evidence for all-or-non regulation of neurotransmitter release: implications for long-term potentiation. *J. Physiol.* **471**: 481–500.

Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. **2006**. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature* **442**: 1042–1045.

Pichat P, Bergis OE, Terranova J-P, Urani A, Duarte C, Santucci V, Gueudet C, Voltz C, Steinberg R, Stemmelin J, Oury-Donat F, Avenet P, Griebel G, Scatton

B. **2007**. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. *Neuropsychopharmacology* **32**: 17–34.

Pidoplichko VI, Noguchi J, Areola OO, Liang Y, Peterson J, Zhang T, Dani JA. **2004**. Nicotinic cholinergic synaptic mechanisms in the ventral tegmental area contribute to nicotine addiction. *Learn. Mem.* **11**: 60–9.

Placzek AN, Zhang TA, Dani JA. **2010**. Nictotinic mechanisms influencing synaptic plasticity in the hippocampus. *Acta Pharmacol. Sin.* **30**: 752–760.

Pontieri FE, Tanda G, Orzi F, Di Chiara G. **1996**. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* **382**: 255–7.

Poorthuis RB, Bloem B, Schak B, Wester J, de Kock CPJ, Mansvelder HD. **2012**. Layer-Specific Modulation of the Prefrontal Cortex by Nicotinic Acetylcholine Receptors. *Cereb. Cortex*.

Popik P, Wrobel M, Bisaga A. **2006**. Reinstatement of morphine-conditioned reward is blocked by memantine. *Neuropsychopharmacology* **31**: 160–70.

Portugal GS, Al-Hasani R, Fakira AK, Gonzalez-Romero JL, Melyan Z, McCall JG, Bruchas MR, Morón J a. **2014**. Hippocampal Long-Term Potentiation Is Disrupted during Expression and Extinction But Is Restored after Reinstatement of Morphine Place Preference. *J. Neurosci.* **34**: 527–38.

Pothuizen HHJ, Zhang WN, Jongen-Rêlo AL, Feldon J, Yee BK. **2004**. Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: A within-subject, within-task comparison of reference and working spatial memory. *Eur. J. Neurosci.* **19**: 705–712.

Pourmotabbed A, Nedaei SE, Mehrabinasab E. **2006**. Assessment of the role of NMDA receptor located in hippocampal CA1 area on the effects of oral morphine dependency on spatial learning and memory in rat. *Physiol. Pharmacol.* **10**: 115–123.

Przybyslawski J, Roullet P, Sara SJ. **1999**. Attenuation of Emotional and Nonemotional Memories after their Reactivation: Role of beta Adrenergic Receptors. *J. Neurosci.* **19**: 6623–6628.

Pych JC, Chang Q, Colon-Rivera C, Haag R, Gold PE. **2005**. Acetylcholine release in the hippocampus and striatum during place and response training. *Learn. Mem.* **12**: 564–72.

Quick MW, Lester R a J. **2002**. Desensitization of neuronal nicotinic receptors. *J. Neurobiol.* **53**: 457–78.

Quirk GJ, Mueller D. **2009**. Neural Mechanisms of Extinction Learning and Retrieval. *Neuropsychopharmacology* **33**: 56–72.

Rada P, Mark GP, Pothos E, Hoebel BG. **1991**. Systemic morphine simultaneously decreases extracellular acetylcholine and increases dopamine in the nucleus accumbens of freely moving rats. *Neuropharmacology* **30**: 1133–6.

Rada P V, Mark GP, Yeomans JJ, Hoebel BG. **2000**. Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating, and drinking. *Pharmacol. Biochem. Behav.* **65**: 375–9.

Rahman S, Engleman E a., Bell RL. **2015**. Nicotinic receptor modulation to treat alcohol and drug dependence. *Front. Neurosci.* **8**: 1–11.

Ran I, Gkogkas CG, Vasuta C, Tartas M, Khoutorsky A, Laplante I, Parsyan A, Nevarko T, Sonenberg N, Lacaille J-C. **2013**. Selective regulation of GluA subunit synthesis and AMPA receptor-mediated synaptic function and plasticity by the translation repressor 4E-BP2 in hippocampal pyramidal cells. *J. Neurosci.* **33**: 1872–86.

Ranaldi R, Kest K, Zellner MR, Lubelski D, Muller J, Cruz Y, Saliba M. **2011**. The effects of VTA NMDA receptor antagonism on reward-related learning and associated c-fos expression in forebrain. *Behav. Brain Res.* **216**: 424–432.

Rapier C, Lunt GG, Wonnacott S. **1990**. Nicotinic modulation of [3H]dopamine release from striatal synaptosomes: pharmacological characterisation. *J. Neurochem.* **54**: 937–45.

Raybuck JD, Gould TJ. **2010**. The role of nicotinic acetylcholine receptors in the medial prefrontal cortex and hippocampus in trace fear conditioning. *Neurobiol. Learn. Mem.* **94**: 353–363.

Rescorla R a, Wagner a R. **1972**. A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. *Class. Cond. II Curr. Res. Theory* **21**: 64–99.

Reus VI, Obach RS, Coe JW, Faessel H, Rollema H, Watsky E, Reeves K. **2007**. Varenicline: new treatment with efficacy in smoking cessation. *Drugs Today (Barc).* **43**: 65–75.

Reynolds IJ. **2001**. [3H](+)MK801 radioligand binding assay at the N-methyl-D-aspartate receptor. *Curr. Protoc. Pharmacol.* **Chapter 1**: Unit 1.20.

Rezayof A, Darbandi N, Zarrindast M-R. **2008**. Nicotinic acetylcholine receptors of the ventral tegmental area are involved in mediating morphine-state-dependent learning. *Neurobiol. Learn. Mem.* **90**: 255–60.

Rezayof A, Golhasani-Keshtan F, Haeri-Rohani A, Zarrindast M-R. **2007**. Morphine-induced place preference: involvement of the central amygdala NMDA receptors. *Brain Res.* **1133**: 34–41.

Rezayof A, Zatali H, Haeri-Rohani A, Zarrindast M-R. **2006**. Dorsal hippocampal muscarinic and nicotinic receptors are involved in mediating morphine reward. *Behav. Brain Res.* **166**: 281–90.

Rezvani AH, Kholdebarin E, Brucato FH, Callahan PM, Lowe DA, Levin ED. **2009**. Effect of R3487/MEM3454, a novel nicotinic alpha7 receptor partial agonist and 5-HT3 antagonist on sustained attention in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **33**: 269–75.

Rezvani AH, Levin ED. **2001**. Cognitive effects of nicotine. *Biol. Psychiatry* **49**: 258–67.

Do Ribeiro Couto B, Aguilar MA, Rodríguez-Arias M, Miñarro J. **2005**. Cross-reinstatement by cocaine and amphetamine of morphine-induced place preference in mice. *Behav. Pharmacol.* **16**: 253–9.

Ribeiro Do Couto B, Aguilar M a, Manzanedo C, Rodríguez-Arias M, Miñarro J. **2005a**. NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. *Brain Res. Bull.* **64**: 493–503.

Ribeiro Do Couto B, Aguilar M a, Rodríguez-Arias M, Miñarro J. **2005b**. Long-lasting rewarding effects of morphine induced by drug primings. *Brain Res.* **1050**: 53–63.

Riga D, Theijs JT, De Vries TJ, Smit AB, Spijker S. **2015**. Social defeat-induced anhedonia: effects on operant sucrose-seeking behavior. *Front. Behav. Neurosci.* **9**: 195.

Rigby PT. **2012**. Synaptic plasticity processes underlying consolidation and reconsolidation of Pavlovian conditioning.

Risinger FO, Oakes R a. **1995**. Nicotine-induced conditioned place preference and conditioned place aversion in mice. *Pharmacol. Biochem. Behav.* **51**: 457–61.

Robbins TW, Ersche KD, Everitt BJ. **2008**. Drug addiction and the memory systems of the brain. *Ann. N. Y. Acad. Sci.* **1141**: 1–21.

Roberts AC, Tomic DL, Parkinson CH, Roeling TA, Cutter DJ, Robbins TW, Everitt BJ. **2007**. Forebrain connectivity of the prefrontal cortex in the marmoset monkey (Callithrix jacchus): an anterograde and retrograde tract-tracing study. *J. Comp. Neurol.* **502**: 86–112.

Roerig B, Nelson D a, Katz LC. **1997**. Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT3 receptors in developing visual cortex. *J. Neurosci.* **17**: 8353–8362.

Rogers JL, See RE. **2007**. Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. *Neurobiol. Learn. Mem.* **87**: 688–692.

Roitman MF, Stuber GD, Phillips PEM, Wightman RM, Carelli RM. **2004**. Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci.* **24**: 1265–1271.

Roitman M, Wheeler R, Wightman R, Carell R. **2008**. Differentiate Rewarding and Aversive Stimuli. *Nat. Neurosci.* **11**: 1376–1377.

Roncarati R, Scali C, Comery TA, Grauer SM, Aschmi S, Bothmann H, Jow B, Kowal D, Gianfriddo M, Kelley C, Zanelli U, Ghiron C, Haydar S, Dunlop J, Terstappen GC. **2009**. Procognitive and neuroprotective activity of a novel alpha7 nicotinic acetylcholine receptor agonist for treatment of neurodegenerative and cognitive disorders. *J. Pharmacol. Exp. Ther.* **329**: 459–68.

Rossi NA, Reid LD. **1976**. Affective states associated with morphine injections. *Physological Psychol.* **4**: 269–274.

Routtenberg A, Lindy J. **1965**. Effects of the availability of rewarding septal and hypothalamic stimulation on bar pressing for food under conditions of deprivation. *J. Comp. Physiol. Psychol.* **60**: 158–61.

Rubboli F, Court JA, Sala C, Morris C, Chini B, Perry E, Clementi F. **1994**. Distribution of nicotinic receptors in the human hippocampus and thalamus. *Eur. J. Neurosci.* **6**: 1596–604.

Sajja RK, Rahman S. **2013**. Nicotinic receptor partial agonists modulate alcohol deprivation effect in C57BL/6J mice. *Pharmacol. Biochem. Behav.* **110**: 161–167.

Sakoori K, Murphy NP. **2005**. Maintenance of conditioned place preferences and aversion in C57BL6 mice: effects of repeated and drug state testing. *Behav. Brain Res.* **160**: 34–43.

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR. **2004**. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol. Pharmacol.* **65**: 1526–35.

Sanchez CJ, Sorg B a. **2001**. Conditioned fear stimuli reinstate cocaine-induced conditioned place preference. *Brain Res.* **908**: 86–92.

Sargent PB. **1993**. The diversity of neuronal nicotinic acetylcholine receptors. *Annu. Rev. Neurosci.* **16**: 403–43.

Sarter M, Kim Y. **2015**. Interpreting chemical neurotransmission in vivo: techniques, time scales, and theories. *ACS Chem. Neurosci.* **6**: 8–10.

Schedel A, Thornton S, Schloss P, Klüter H, Bugert P. **2011**. Human platelets express functional alpha7-nicotinic acetylcholine receptors. *Arterioscler. Thromb. Vasc. Biol.* **31**: 928–34.

Schenk S, Lacelle G, Gorman K, Amit Z. **1987**. Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. *Neurosci. Lett.* **81**: 227–31.

Schlussman SD, Zhang Y, Hsu NM, Allen JM, Ho A, Kreek MJ. **2008**. Heroin-induced locomotor activity and conditioned place preference in C57BL/6J and 129P3/J mice. *Neurosci. Lett.* **440**: 284–8.

Schmidt HD, Anderson SM, Famous KR, Kumaresan V, Pierce RC. **2005a**. Anatomy and pharmacology of cocaine priming-induced reinstatement of drug seeking. *Eur. J. Pharmacol.* **526**: 65–76.

Schmidt HD, Anderson SM, Famous KR, Kumaresan V, Pierce RC. **2005b**. Anatomy and pharmacology of cocaine priming-induced reinstatement of drug seeking. *Eur. J. Pharmacol.* **526**: 65–76.

Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA. **2002**. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc. Natl. Acad. Sci. U. S. A.* **99**: 13902–7.

Schultz W. **2000**. Multiple reward signals in the brain. *Nat. Rev. Neurosci.* **1**: 199–207.

Schultz W. **2001**. Reward signaling by dopamine neurons. *Neuroscientist* **7**: 293–302.

Schultz W. **2007**. Behavioral dopamine signals. *Trends Neurosci.* **30**: 203–10.

Schurr A. **1981**. Brain Slice Preparation In Electrophysiology. *David Kopf instruments* **15**.

Seamans JK, Floresco SB, Phillips AG. **1995**. Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behav. Neurosci.* **109**: 1063–73.

Séguéla P, Wadiche J, Dineley-Miller K, Dani J a, Patrick JW. **1993**. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. *J. Neurosci.* **13**: 596–604.

Sepulveda MJ, Hernandez L, Rada P, Tucci S, Contreras E. **1998**. Effect of precipitated withdrawal on extracellular glutamate and aspartate in the nucleus accumbens of chronically morphine-treated rats: an in vivo microdialysis study. *Pharmacol. Biochem. Behav.* **60**: 255–62.

Sharf R, Ranaldi R. **2006**. Blockade of muscarinic acetylcholine receptors in the ventral tegmental area disrupts food-related learning in rats. *Psychopharmacology (Berl).* **184**: 87–94.

Sharma G, Vijayaraghavan S. **2002**. Nicotinic receptor signaling in nonexcitable cells. *J. Neurobiol.* **53**: 524–34.

Sharma G, Vijayaraghavan S. **2003**. Modulation of presynaptic store calcium induces release of glutamate and postsynaptic firing. *Neuron* **38**: 929–939.

Shen J, Yakel JL. **2009**. Nicotinic acetylcholine receptor-mediated calcium signaling in the nervous system. *Acta Pharmacol. Sin.* **30**: 673–80.

Shepherd JD, Huganir RL. **2007**. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu. Rev. Cell Dev. Biol.* **23**: 613–43.

Shiffman S, Balabanis M. **1992**. Do Drinking and Smoking Go Together ? *Alcohol Health Res. World* **20**: 1990–1993.

Shippenberg TS, Bals-Kubik R, Herz a. **1993**. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J. Pharmacol. Exp. Ther.* **265**: 53–59.

Shoblock JR, Wichmann J, Maidment NT. **2005**. The effect of a systemically active ORL-1 agonist, Ro 64-6198, on the acquisition, expression, extinction, and reinstatement of morphine conditioned place preference. *Neuropharmacology* **49**: 439–46.

Singer S, Rossi S, Verzosa S, Hashim A, Lonow R, Cooper T, Sershen H, Lajtha A. **2004**. Nicotine-induced changes in neurotransmitter levels in brain areas associated with cognitive function. *Neurochem. Res.* **29**: 1779–92.

Siok CJ, Rogers J a, Kocsis B, Hajós M. **2006**. Activation of alpha7 acetylcholine receptors augments stimulation-induced hippocampal theta oscillation. *Eur. J. Neurosci.* **23**: 570–4.

Skinner B. **1938**. The behavior of organisms an experimental analysis,. New York London: D. Appleton-Century Company Incorporated.

Smith JE, Guerin GF, Co C, Barr TS, Lane JD. **1985**. Effects of 6-OHDA lesions of the central medial nucleus accumbens on rat intravenous morphine self-administration. *Pharmacol. Biochem. Behav.* **23**: 843–9.

Smith GP, Strohmayer AJ, Reis DJ. **1972**. Effect of Lateral Hypothalamic Injections of 6-Hydroxydopamine on Food and Water Intake in Rats. *Nature* **235**: 27–29.

Sobell LC, Sobell MB, Kozlowski LT, Toneatto T. **1990**. Alcohol or tobacco research versus alcohol and tobacco research. *Br. J. Addict.* **85**: 263–9.

Söderpalm B, Ericson M, Olausson P, Blomqvist O, Engel J a. **2000**. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav. Brain Res.* **113**: 85–96.

Solinas M, Scherma M, Fattore L, Stroik J, Wertheim C, Tanda G, Fratta W, Goldberg SR. **2007**. Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. *J. Neurosci.* **27**: 5615–5620.

Sombers LA, Beyene M, Carelli RM, Wightman RM. **2009**. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. *J. Neurosci.* **29**: 1735–42.

Squire L, Barondes S. **1972**. Macromolecules and Behavior: Chapter 5 Inhibitors of cerebral protein or RNA synthesis and memory (J Gaito, Ed.). Boston, MA: Springer US.

Squire L, Davis H. **1981**. The Pharmacology of Memory: A Neurobiological Perspective. *Ann. Rev. Pharmacol. Toxicol.*: 323–356.

Squire L, Smith G, Barondes S. **1973**. Cycloheximide affects Memory within Minutes after the Onset of Training. *Nature* **242**: 201–202.

Steensland P, Simms J a, Holgate J, Richards JK, Bartlett SE. **2007**. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc. Natl. Acad. Sci. U. S. A.* **104**: 12518–12523.

Stone CA, Torchiana ML, Navarro A, Beyer KH. **1956**. Ganglionic blocking properties of 3-methylaminoisocamphane hydrochloride (mecamylamine): a secondary amine. *J. Pharmacol. Exp. Ther.* **117**: 169–183.

Sucher NJ, Awobufuyi M, Choi YB, Lipton S a. **1996**. NMDA receptors: From genes to channels. *Trends Pharmacol. Sci.* **17**: 348–355.

Sudweeks SN, Yakel JL. **2000**. Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons. *J. Physiol.* **527 Pt 3**: 515–528.

Suh HW, Song DK, Choi SR, Chung KM, Kim YH. **1996**. Nicotine enhances morphine- and beta-endorphin-induced antinociception at the supraspinal level in the mouse. *Neuropeptides* **30**: 479–84.

Swanson LW, Wyss JM, Cowan WM. **1978**. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J. Comp. Neurol.* **181**: 681–715.

Sweatt JD. **2001**. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* **76**: 1–10.

Tang A-H, Karson MA, Nagode DA, McIntosh JM, Uebele VN, Renger JJ, Klugmann M, Milner TA, Alger BE. **2011a**. Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca^{2+} channels and Ca^{2+} release from stores. *J. Neurosci.* **31**: 13546–61.

Tang A-H, Karson MA, Nagode DA, McIntosh JM, Uebele VN, Renger JJ, Klugmann M, Milner TA, Alger BE. **2011b**. Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca^{2+} channels and Ca^{2+} release from stores. *J. Neurosci.* **31**: 13546–61.

Tannenholz L, Jimenez JC, Kheirbek MA. **2014**. Local and regional heterogeneity underlying hippocampal modulation of cognition and mood. *Front. Behav. Neurosci.* **8**: 147.

Taube JS. **2007**. The head direction signal: origins and sensory-motor integration. *Annu. Rev. Neurosci.* **30**: 181–207.

Taylor JR, Robbins TW. **1984**. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl).* **84**: 405–12.

The Global Drug Survey. **2014**. *http://www.globaldrugsurvey.com/facts-figures/the-global-drug-survey-2014-findings/*.

Thomas MJ, Malenka RC. **2003**. Synaptic plasticity in the mesolimbic dopamine system. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **358**: 815–819.

Tietje KR, Anderson DJ, Bitner RS, Blomme EA, Brackemeyer PJ, Briggs CA, Browman KE, Bury D, Curzon P, Drescher KU, Frost JM, Fryer RM, Fox GB, Gronlien JH, Håkerud M, Gubbins EJ, Halm S, Harris R, Helfrich RJ, Kohlhaas KL, Law D, Malysz J, Marsh KC, Martin RL, Meyer MD, Molesky AL, Nikkel AL, Otte S, Pan L, Puttfarcken PS, Radek RJ, Robb HM, Spies E, Thorin-Hagene K, Waring JF, Ween H, Xu H, Gopalakrishnan M, Bunnelle WH. **2008**. Preclinical characterization of A-582941: a novel alpha7 neuronal nicotinic receptor agonist with broad spectrum cognition-enhancing properties. *CNS Neurosci. Ther.* **14**: 65–82.

Timmermann DB, Grønlien JH, Kohlhaas KL, Nielsen EØ, Dam E, Jørgensen TD, Ahring PK, Peters D, Holst D, Christensen JK, Chrsitensen JK, Malysz J, Briggs CA, Gopalakrishnan M, Olsen GM. **2007**. An allosteric modulator of the alpha7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo. *J. Pharmacol. Exp. Ther.* **323**: 294–307.

Tinsley CJ, Fontaine-Palmer NS, Vincent M, Endean EPE, Aggleton JP, Brown MW, Warburton EC. **2011**. Differing time dependencies of object recognition memory impairments produced by nicotinic and muscarinic cholinergic antagonism in perirhinal cortex. *Learn. Mem.* **18**: 484–492.

Totterdell S, Smith AD. **1989**. Convergence of hippocampal and dopaminergic input onto identified neurons in the nucleus accumbens of the rat. *J. Chem. Neuroanat.* **2**: 285–98.

Triller A, Choquet D. **2008**. New concepts in synaptic biology derived from single-molecule imaging. *Neuron* **59**: 359–74.

Tzschentke TM, Schmidt WJ. **2003**. Glutamatergic mechanisms in addiction. *Mol. Psychiatry* **8**: 373–382.

Udakis M, Rupawala M, Wonnacott S, Bailey CP. **2013**. Regulation of synpatic plasticity in the mouse prelimbic cortex by α7 nicotinic acetylcholine receptors. Proceedings of the British Pharmacological Society.Vol11, Issue 3.

Ullian EM, McIntosh JM, Sargent PB. **1997**. Rapid synaptic transmission in the avian ciliary ganglion is mediated by two distinct classes of nicotinic receptors. *J. Neurosci.* **17**: 7210–7219.

Unwin N. **1995**. Acetylcholine receptor channel imaged in the open state. *Nature* **373**: 37–43.

Valenti O, Lodge DJ, Grace AA. **2011**. Aversive stimuli alter ventral tegmental area dopamine neuron activity via a common action in the ventral hippocampus. *J. Neurosci.* **31**: 4280–9.

Varanda W a, Aracava Y, Sherby SM, VanMeter WG, Eldefrawi ME, Albuquerque EX. **1985**. The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol. Pharmacol.* **28**: 128–137.

Verrico CD, Mahoney JJ, Thompson-Lake DGY, Bennett RS, Newton TF, De La Garza R. **2014**. Safety and efficacy of varenicline to reduce positive subjective effects produced by methamphetamine in methamphetamine-dependent volunteers. *Int. J. Neuropsychopharmacol.* **17**: 223–33.

Vertes RP. **2006**. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience* **142**: 1–20.

Vicens P, Ribes D, Torrente M, Domingo JL. **2011**. Behavioral effects of PNU-282987, an alpha7 nicotinic receptor agonist, in mice. *Behav. Brain Res.* **216**: 341–8.

Vihavainen T, Mijatovic J, Piepponen TP, Tuominen RK, Ahtee L. **2006**. Effect of morphine on locomotor activity and striatal monoamine metabolism in nicotine-withdrawn mice. *Behav. Brain Res.* **173**: 85–93.

Vihavainen T, Piltonen M, Tuominen RK, Korpi ER, Ahtee L. **2008a**. Morphine-nicotine interaction in conditioned place preference in mice after chronic nicotine exposure. *Eur. J. Pharmacol.* **587**: 169–74.

Vihavainen T, Relander TRA, Leiviskä R, Airavaara M, Tuominen RK, Ahtee L, Piepponen TP. **2008b**. Chronic nicotine modifies the effects of morphine on extracellular striatal dopamine and ventral tegmental GABA. *J. Neurochem.* **107**: 844–54.

Vogt BA, Miller MW. **1983**. Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices. *J. Comp. Neurol.* **216**: 192–210.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Hitzemann R, Chen AD, Dewey SL, Pappas N. **1997**. Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature* **386**: 830–3.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Childress A-R, Jayne M, Ma Y, Wong C. **2006**. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J. Neurosci.* **26**: 6583–8.

Wada K, Ballivet M, Boulter J, Connolly J, Wada E, Deneris ES, Swanson LW, Heinemann S, Patrick J. **1988**. Functional expression of a new pharmacological subtype of brain nicotinic acetylcholine receptor. *Science* **240**: 330–4.

Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW. **1989**. Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J. Comp. Neurol.* **284**: 314–35.

Wallenstein G V, Hasselmo ME. **1997**. GABAergic modulation of hippocampal population activity: sequence learning, place field development, and the phase precession effect. *J. Neurophysiol.* **78**: 393–408.

Walters CL, Brown S, Changeux J-P, Martin B, Damaj MI. **2006**. The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology (Berl).* **184**: 339–44.

Wand G. **2005**. The anxious amygdala: CREB signaling and predisposition to anxiety and alcoholism. *J. Clin. Invest.* **115**: 2697–9.

Wang Z, Edwards JG, Riley N, Provance DW, Karcher R, Li X-D, Davison IG, Ikebe M, Mercer JA, Kauer JA, Ehlers MD. **2008**. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell* **135**: 535–48.

Wang B, Luo F, Zhang WT, Han JS. **2000**. Stress or drug priming induces reinstatement of extinguished conditioned place preference. *Neuroreport* **11**: 2781–4.

Wang J, Wu X, Li C, Wei J, Jiang H, Liu C, Yu C, Carlson S, Hu X, Ma H, Duan W, Ma Y. **2011**. Effect of morphine on conditioned place preference in rhesus monkeys. *Addict. Biol.*

Ward JM, Cockcroft VB, Lunt GG, Smillie FS, Wonnacott S. **1990**. Methyllycaconitine: a selective probe for neuronal alpha-bungarotoxin binding sites. *FEBS Lett.* **270**: 45–48.

Weingartner H. **1978**. Human state dependent learning. In: Drug discrimination and state dependent learning (T Ho, D Richards, and D Chute, Eds.). Academic Press: New York.

Wessler I, Kirkpatrick CJ. **2008**. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br. J. Pharmacol.* **154**: 1558–71.

Westerink BH, Kwint HF, deVries JB. **1996**. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J. Neurosci.* **16**: 2605–11.

White NM. **1996**. Addictive drugs as reinforcers: multiple partial actions on memory systems. *Addiction* **91**: 921–49; discussion 951–65.

White NM, McDonald RJ. **2002**. Multiple parallel memory systems in the brain of the rat. *Neurobiol. Learn. Mem.* **77**: 125–184.

Whiteaker P, McIntosh JM, Luo S, Collins a C, Marks MJ. **2000**. 125I-alpha-conotoxin MII identifies a novel nicotinic acetylcholine receptor population in mouse brain. *Mol. Pharmacol.* **57**: 913–925.

Whiting P, Lindstrom J. **1987**. Affinity labelling of neuronal acetylcholine receptors localizes acetylcholine-binding sites to their beta-subunits. *FEBS Lett.* **213**: 55–60.

Wiens F, Zitzmann A, Lachance M-A, Yegles M, Pragst F, Wurst FM, von Holst D, Guan SL, Spanagel R. **2008**. Chronic intake of fermented floral nectar by wild treeshrews. *Proc. Natl. Acad. Sci.* **105**: 10426–10431.

Wilkes S. **2008**. The use of bupropion SR in cigarette smoking cessation. *Int. J. COPD* **3**: 45–53.

Wilkie GI, Hutson PH, Stephens MW, Whiting P, Wonnacott S. **1993**. Hippocampal nicotinic autoreceptors modulate acetylcholine release. *Biochem. Soc. Trans.* **21**: 429–31.

Williams MJ, Adinoff B. **2009**. The Role of Acetylcholine in Cocaine Addiction. *Neuropsychopharmacology* **33**: 1779–1797.

Wise R a. **2004**. Dopamine, learning and motivation. *Nat. Rev. Neurosci.* **5**: 483–94.

Wise RA. **2009**. Roles for nigrostriatal--not just mesocorticolimbic--dopamine in reward and addiction. *Trends Neurosci.* **32**: 517–24.

Wise RA, Bozarth MA. **1987**. A Psychomotor Stimulant Theory of Addiction. **94**: 469–492.

Wise RA, Schwartz H V. **1981**. Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol. Biochem. Behav.* **15**: 655–6.

Witter MP, Amaral DG. **1991**. Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J. Comp. Neurol.* **307**: 437–59.

Witter MP, Groenewegen HJ. **1984**. Laminar origin and septotemporal distribution of entorhinal and perirhinal projections to the hippocampus in the cat. *J. Comp. Neurol.* **224**: 371–85.

Wonnacott S. **1997**. Presynaptic nicotinic ACh receptors. *Trends Neurosci.* **20**: 92–8.

Wonnacott S, Sidhpura N, Balfour DJK. **2005**. Nicotine: from molecular mechanisms to behaviour. *Curr. Opin. Pharmacol.* **5**: 53–9.

Woodcock EA, Lundahl LH, Stoltman JJK, Greenwald MK. **2015**. Progression to regular heroin use: examination of patterns, predictors, and consequences. *Addict. Behav.* **45**: 287–93.

Woodruff-Pak DS. **2003**. Mecamylamine reversal by nicotine and by a partial alpha7 nicotinic acetylcholine receptor agonist (GTS-21) in rabbits tested with delay eyeblink classical conditioning. *Behav. Brain Res.* **143**: 159–67.

Woolf NJ, Butcher LL. **1986**. Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. *Brain Res. Bull.* **16**: 603–37.

Wouda J a, Riga D, De Vries W, Stegeman M, van Mourik Y, Schetters D, Schoffelmeer ANM, Pattij T, De Vries TJ. **2011**. Varenicline attenuates cue-induced relapse to alcohol, but not nicotine seeking, while reducing inhibitory response control. *Psychopharmacology (Berl).* **216**: 267–77.

Xia Y, Portugal GS, Fakira AK, Melyan Z, Neve R, Lee HT, Russo SJ, Liu J, Morón JA. **2011**. Hippocampal GluA1-containing AMPA receptors mediate context-dependent sensitization to morphine. *J. Neurosci.* **31**: 16279–91.

Xu Y, Lv X-F, Cui C-L, Ge F-F, Li Y-J, Zhang H-L. **2012**. Essential role of NR2B-containing NMDA receptor-ERK pathway in nucleus accumbens shell in morphine-associated contextual memory. *Brain Res. Bull.* **89**: 22–30.

Yakel JL. **2012**. Nicotinic ACh receptors in the hippocampus: role in excitability and plasticity. *Nicotine Tob. Res.* **14**: 1249–57.

Yakel JL, Shao Z. **2004**. Functional and molecular characterization of neuronal nicotinic ACh receptors in rat hippocampal interneurons. *Prog. Brain Res.* **145**: 95–107.

Yang X, Criswell HE, Breese GR. **1996**. Nicotine-induced inhibition in medial septum involves activation of presynaptic nicotinic cholinergic receptors on gamma-aminobutyric acid-containing neurons. *J. Pharmacol. Exp. Ther.* **276**: 482–9.

Yeomans J, Baptista M. **1997**. Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain-stimulation reward. *Pharmacol. Biochem. Behav.* **57**: 915–921.

Yeomans JS, Mathur a, Tampakeras M. **1993**. Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. *Behav. Neurosci.* **107**: 1077–1087.

Yoo J-H, Yang E-M, Cho J-H, Lee J-H, Jeong SM, Nah S-Y, Kim H-C, Kim K-W, Kim S-H, Lee S-Y, Jang C-G. **2006**. Inhibitory effects of berberine against morphine-induced locomotor sensitization and analgesic tolerance in mice. *Neuroscience* **142**: 953–61.

You Z-B, Wang B, Zitzman D, Wise RA. **2008**. Acetylcholine release in the mesocorticolimbic dopamine system during cocaine seeking: conditioned and unconditioned contributions to reward and motivation. *J. Neurosci.* **28**: 9021–9.

Young JW, Geyer MA. **2013**. Evaluating the role of the alpha-7 nicotinic acetylcholine receptor in the pathophysiology and treatment of schizophrenia. *Biochem. Pharmacol.* **86**: 1122–32.

Zachariou V, Caldarone BJ, Weathers-Lowin a, George TP, Elsworth JD, Roth RH, Changeux JP, Picciotto MR. **2001**. Nicotine receptor inactivation decreases sensitivity to cocaine. *Neuropsychopharmacology* **24**: 576–89.

Zanetti L, De Kerchove D'Exaerde A, Zanardi A, Changeux JP, Picciotto MR, Zoli M. **2006**. Inhibition of both alpha7* and beta2* nicotinic acetylcholine receptors is necessary to prevent development of sensitization to cocaine-elicited increases in extracellular dopamine levels in the ventral striatum. *Psychopharmacology (Berl).* **187**: 181–188.

Zarrindast MR, Faraji N, Rostami P, Sahraei H, Ghoshouni H. **2003**. Cross-tolerance between morphine- and nicotine-induced conditioned place preference in mice. *Pharmacol. Biochem. Behav.* **74**: 363–9.

Zarrindast MR, Rezayof A. **2004.** Morphine state-dependent learning: sensitization and interaction with dopamine receptors. *European Journal of Pharmacology.* **497(2)**: 197-204.

Zellner MR, Kest K, Ranaldi R. **2009**. NMDA receptor antagonism in the ventral tegmental area impairs acquisition of reward-related learning. *Behav. Brain Res.* **197**: 442–449.

Zhang ZW, Coggan JS, Berg DK. **1996**. Synaptic currents generated by neuronal acetylcholine receptors sensitive to alpha-bungarotoxin. *Neuron* **17**: 1231–1240.

Zhang M, Wang YT, Vyas DM, Neuman RS, Bieger D. **1993**. Nicotinic cholinoceptor-mediated excitatory postsynaptic potentials in rat nucleus ambiguus. *Exp. brain Res.* **96**: 83–8.

Zhou W, Liu H, Zhang F, Tang S, Zhu H, Lai M, Kalivas PW. **2007**. Role of acetylcholine transmission in nucleus accumbens and ventral tegmental area in heroin-seeking induced by conditioned cues. *Neuroscience* **144**: 1209–1218.

Zhu H, Jang CG, Ma T, Oh S, Rockhold RW, Ho IK. **1999**. Region specific expression of NMDA receptor NR1 subunit mRNA in hypothalamus and pons following chronic morphine treatment. *Eur. J. Pharmacol.* **365**: 47–54.

Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R. **2002**. Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* **110**: 443–55.

Zoli M, Jansson A, Syková E, Agnati LF, Fuxe K. **1999**. Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol. Sci.* **20**: 142–150.

Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C. **2002**. Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J. Neurosci.* **22**: 8785–9.

Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, Darvas M, Kim MJ, Mizumori SJY, Paladini CA, Phillips PEM, Palmiter RD. **2009**. Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 7281–8.